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Phylogenetic reassessment of the coelomycete genus *Harknessia* and its teleomorph *Wuestneia* (*Diaporthales*), and the introduction of *Apoharknessia* gen. nov.

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Abstract: During routine surveys for microfungi from the Fynbos of the Cape Floral Kingdom in South Africa, isolates of several *Harknessia* species were collected. Additional isolates of *Harknessia* spp. were collected from *Eucalyptus* leaves in South Africa, as well as elsewhere in the world where this crop is grown. Interspecific relationships of *Harknessia* species were inferred based on partial sequence of the internal transcribed spacer (ITS) nuclear ribosomal DNA (nrDNA), as well as the β-tubulin and calmodulin genes. From these data, three new species are described, namely *H. globispora* from *Eucalyptus*, *H. protearum* from *Leucadendron* and *Leucospermum*, and *H. capensis* from *Brabejum stellatifolium* and *Eucalyptus* sp. Furthermore, based on large subunit nrDNA sequence data, *Harknessia* is shown to be heterogeneous, and a new genus, *Apoharknessia,* is introduced for *A. insueta*, which is distinguished from *H. eucalypti*, the type species of *Harknessia*, by having an apical conidial appendage. A morphologically similar genus, *Dwiroopa*, which is characterized by several prominent germ slits along the sides of its conidia, is shown to cluster basal to *Harknessia*. Species of *Harknessia*, and their teleomorphs accommodated in *Wuestneia*, are shown to represent an undescribed family in the *Diaporthales*, as is *Apoharknessia*, for which no teleomorph is known.

Taxonomic novelties: *Apoharknessia* Crous & S. Lee gen. nov., *A. insueta* (B. Sutton) Crous & S. Lee comb. nov., *Harknessia capensis* S. Lee & Crous sp. nov., *Harknessia globispora* Crous & S. Lee sp. nov., *Harknessia protearum* S. Lee & Crous sp. nov.
Key words: biodiversity, *Diaporthales*, *Dwiroopa*, *Eucalyptus*, fynbos, *Harknessia*, *Proteaceae*, saprobic fungi.

INTRODUCTION

Species of the coelomycete genus *Harknessia* Cooke are characterised by stromatic to pycnidioid conidiomata, and darkly pigmented conidia with tube-shaped basal appendages, longitudinal striations, and rhexolytic sessesion. Members of this genus occur worldwide as either plant pathogens or saprobes (Sankaran *et al.* 1995, Yuan *et al.* 2000, Crous & Rogers 2001, Farr & Rossman 2001). The genus has been revised several times in recent years (Sutton 1971, Nag Raj & DiCosmo 1981, Nag Raj 1993). Since 1993, a further 11 species have been added to the genus, resulting in 37 species in total (Crous *et al.* 1989, 1993, Furlanetto & Dianese 1998, Swart *et al.* 1998, Yuan *et al.* 2000, Crous & Rogers 2001, Farr & Rossman 2001). Species of *Harknessia* occur on leaves and twigs of various gymnosperm and dicotyledonous hosts. The genus *Eucalyptus* L'Herit. (*Myrtaceae*) is particularly rich in *Harknessia* species, and is currently a host for up to 17 species.

Teleomorphs of *Harknessia* are known to reside in *Wuestneia* Auersw. ex Fuckel (*Melanconidaceae*, *Diaporthales*) (Barr 1990, Kirk *et al.* 2001, Eriksson *et al.* 2003). The genus has recently been treated by Reid and Booth (1989), who described perithecia as being stromatic, producing asci with deliquescent stalks, and monosporous, hyaline, ellipsoidal to inequilateral ascospores. Seven of the 13 *Wuestneia* species known to date have been linked to *Harknessia* anamorphs (Reid and Booth 1989, Sutton & Pascoe 1989, Crous *et al.* 1993, Crous & Rogers 2001).

During a study focusing on collecting and describing the saprobic microfungi occurring in the Fynbos of the Cape Floral Kingdom of South Africa (Taylor *et al.* 2001), several collections of *Harknessia* species were made from a variety of host plants. Additional collections were also made from *Eucalyptus* leaves in South Africa, as well as elsewhere as part of a study focusing on foliicolous pathogens of *Myrtaceae*. While attempting to name these collections, a paper circumscribing the relationships of conventionally known members of the *Diaporthales* by Castlebury *et al.* (2002) revealed that, based on large subunit (LSU) nrDNA sequences, members of the *Wuestneia* clade grouped outside of the *Melanconidaceae*. Consequently, we decided to also investigate the familiar status of the *Wuestneia*/*Harknessia* clade in the *Diaporthales*. To address these questions, three genes were sequenced to resolve species (ITS, β-tubulin,

calmodulin), while data from the LSU were used to resolve families within the *Diaporthales*.

MATERIALS AND METHODS

Isolates and light microscopy

Leaf or twig litters of members of *Proteaceae* were collected from nature reserves and a national park in the Western Cape province of South Africa over a two-year period (2000–2001). *Eucalyptus* leaves were collected randomly from various countries (Table 1). Air-dried samples were incubated in moisture chambers for 2–3 d before examination. Single-spore isolation was carried out and cultures were established on malt extract agar (MEA; Biolab, Midrand, Johannesburg) containing 2% malt extract, supplemented with 0.04 g/L streptomycin sulphate. Cultural characteristics were determined in triplicate from MEA plates after 5–8 d of incubation at 25 °C in the dark, and colours determined according to Rayner (1970). Differential interference contrast was employed for the general observation except for the determination of colour, in which case bright field was adopted. Measurements and photographs of characteristic structures were made from structures mounted in lactic acid, while mounts were made in water to observe mucous appendages. The 95 % confidence intervals were derived from 30 observations, with the extremes given in parentheses. Pieces of plant tissue bearing conidiomata were soaked in water overnight and mounted with Jung tissue freezing medium™ (Leica Instruments, Germany) for microsection. Sections of conidiomata were made on a Leica CM1100 Cryostat microtome and mounted in 70 % aqueous lactic acid, which was later replaced by lactophenol. Photographic images were captured with a Nikon Digital Camera DXM 1200 on a Nikon Eclipse E600 light microscope or a Nikon SMZ800 dissecting microscope. Herbarium specimens are deposited in the National Collection of Fungi, Pretoria (PREM) in South Africa, or the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands, and reference strains are maintained at the CBS or the Department of Plant Pathology, University of Stellenbosch (STE-U).

Sequencing and phylogenetic analyses

Thirty-five isolates of *Harknessia* and *Harknessia*-like taxa were sequenced and subjected to phylogenetic analyses. Detailed information on substrata, voucher and accession numbers is provided in Table 1. The isolation protocol of Lee & Taylor (1990) was used to extract genomic DNA from fungal mycelia grown on MEA. The primers ITS1 (White *et al.* 1990) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rRNA operon using the PCR conditions recommended by the authors and spanning the 3' end of the 18S rRNA gene, the internal spacers, the 5.8S rRNA gene and a part of the 5' end of the 28S rRNA gene. Part of the β-tubulin gene was amplified using primers T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). The primers CAL-228F and CAL-737R (Carbone & Kohn 1999) were used to amplify part of the calmodulin gene. PCR conditions were the same for all regions, except for the $MgCl_2$ concentration, which was 1.5 mM for both calmodulin and the partial nuclear rRNA operon and 1.0 mM for the β-tubulin region. PCR products were separated by electrophoresis at 80 V for 1 h in a 0.8 % (w/v) agarose gel in 0.5× TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, U.K.) following ethidium bromide staining. The amplification products were purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech Europe GmbH, Germany). The purified products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI PRISM Big Dye Terminator v. 3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) containing AmpliTaq DNA Polymerase. For the large subunit gene, the internal primers LR0R (Rehner & Samuels 1994) and LR16 (Moncalvo *et al.* 1993) were additionally used to ensure good quality sequences over the entire length of the amplicon. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

The sequences were assembled and added to the outgroup and some GenBank sequences for related fungi using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002), and manual adjustments for improvement were made by eye where necessary. The phylogenetic analyses of sequence data were done in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2000) and consisted of neighbour-joining analysis with the uncorrected ("p"), the Jukes-Cantor and the Kimura 2-parameter substitution model in PAUP. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. For parsimony analysis, alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull 1993).

Table 1. List of species sequenced in this study.

Fungal species	Cultures[1]	Collection locality	Host plants	GenBank accession no.			
				ITS	β-tubulin	Calmodulin	LSU
Apoharknessia insueta	CBS 114575 = STE-U 10947	Colombia	*Eucalyptus* sp.				AY720813
	CBS 111377 = STE-U 1451	Brazil	*E. pellita*				AY720814
Dwiroopa lythri	CBS 109755[2]	U.S.A.	*Lythrum salicaria*				AF408364
Harknessia capensis	CBS 115061 = STE-U 10867	South Africa	*Eucalyptus* sp.	AY720718	AY720750	AY720781	AY720815
	CBS 111829[2] = STE-U 5468	South Africa	*Brabejum stellatifolium*	AY720719	AY720751	AY720782	AY720816
H. "fusiformis"	STE-U 10488	South Africa	*Eucalyptus* sp.	AY720720	AY720752	AY720783	AY720817
H. fusiformis	STE-U 295[2]	South Africa	*Eucalyptus* sp.	AY720721	AY720753	AY720784	AY720818
H. globispora	CBS 111578[2] = STE-U 3710	Portugal	*E. globulus*	AY720722	AY720754	AY720785	AY720819
H. hawaiiensis	CBS 114811 = STE-U 10957	Colombia	*Eucalyptus* sp.	AY720723	AY720755	AY720786	AY720820
	CBS 115650 = STE-U 10960	Colombia	*Eucalyptus* sp.	AY720724	AY720756	AY720787	AY720821
	CBS 110728 = STE-U 113	South Africa	*E. viminalis*	AY720725	AY720757	AY720788	AY720822
	CBS 111122 = STE-U 180	South Africa	*E. grandis*	AY720726	AY720758	AY720789	AY720823
H. leucospermi	CBS 775.97[2] = STE-U 1373	South Africa	*Leucospermum* sp.	AY720727	AY720759	AY720790	AY720824
	STE-U 2849	South Africa	*Leucospermum* sp.	AY720728	AY720760	AY720791	AY720825
	STE-U 5400	South Africa	*Leuco. praecox*	AY720729	AY720761	AY720792	AY720826
	CBS 112620 = STE-U 5403	South Africa	Unidentified *Proteaceae*	AY720730	AY720762	AY720793	AY720827
	CBS 112619 = STE-U 5404	South Africa	*Protea laurifolia*	AY720731	AY720763	AY720794	
H. protearum	CBS 112618[2] = STE-U 5405	South Africa	*Leuco. oleaefolium*	AY720732	AY720764	AY720795	AY720828
	CBS 112617 = STE-U 5406	South Africa	*Leucospermum* sp.	AY720733	AY720765	AY720796	AY720829
	CBS 112616 = STE-U 5407	South Africa	*Leucadendron* sp.	AY720734	AY720766	AY720797	AY720830
	CBS 111830 = STE-U 5469	South Africa	*Leucospermum* sp.	AY720735	AY720767	AY720798	AY720831
	CBS 111831 = STE-U 5470	South Africa	*Leuca. conocarpodendron*	AY720736	AY720768	AY720799	AY720832
H. renispora	CBS 153.71	Australia	*Melaleuca lanceolata*	AY720737	AY720769	AY720800	AY720833
H. syzygii	STE-U 184[2]	South Africa	*Syzygium cordatum*	AY720738	AY720770	AY720801	AY720834
H. "uromycoides"	STE-U 108	South Africa	*Eucalyptus* sp.	AY720739	AY720771	AY720802	
	STE-U 10843	Italy	*Eucalyptus* sp.	AY720740	AY720772	AY720803	
H. weresubiae	CBS 113075 = STE-U 5106	South Africa	*Eucalyptus* sp.	AY720741	AY720773	AY720804	AY720835
	STE-U 5107	South Africa	*Eucalyptus* sp.	AY720742	AY720774	AY720805	AY720836
	STE-U 5108	South Africa	*Eucalyptus* sp.	AY720743	AY720775	AY720806	AY720837
	STE-U 5109	South Africa	*Eucalyptus* sp.	AY720744	AY720776	AY720807	AY720838
Wuestneia epispora (?*H. eucalypti*)	CBS 342.97	Australia	*E. regnans*	AY720745	AY720777	AY720808	AF408363
W. eucalyptorum (*H. eucalyptorum*)	CBS 113620	Spain	*Eucalyptus* sp.	AY720746	AY720778	AY720809	AY720839
	STE-U 85[2]	South Africa	*E. tereticornis*	AY720747	AY720779	AY720810	AY720840
W. karwarrae (*H. kar warrae*)	CBS 115648 = STE-U 10928	New Zealand	*E. botryoides*	AY720748	AY720780	AY720811	AY720841
W. molokaiensis (*H. molokaiensis*)	CBS 114877[2] = STE-U 3797	USA (Hawaii)	*E. robusta*	AY720749	AY579335	AY720812	AY720842

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa. [2]Ex-type cultures.

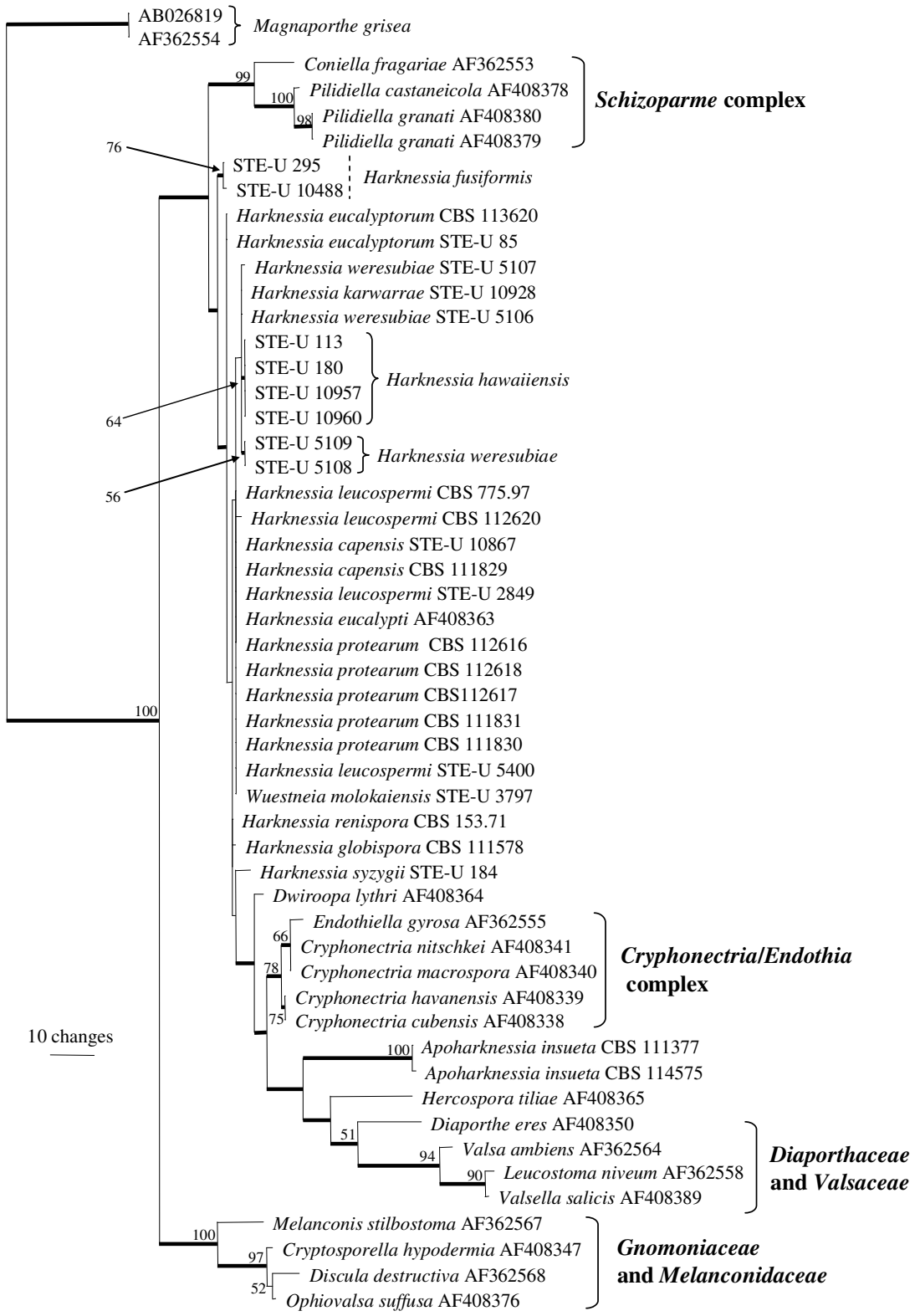


Fig. 1. One of 108 most parsimonious trees obtained from a parsimony analysis using the large subunit rRNA gene sequence data (TL = 295 steps, CI = 0.624, RI = 0.795, RC = 0.496). The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Two sequences of *Magnaporthe grisea* (GenBank accessions AB026819 and AF362554) were included as outgroups.

Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC). The resulting trees were printed with TreeView v. 1.6.6 (Page 1996).

RESULTS

Phylogenetic analysis

For the large ribosomal subunit gene, approximately 850 bases were determined for the isolates as indicated in Table 1. The manually adjusted alignment contained 52 taxa (including the two outgroups) and 860 characters including alignment gaps (TreeBASE accession S1149). Of the 643 characters used in the phylogenetic analysis, 122 were parsimony-informative, 25 were variable and parsimony-uninformative, and 496 were constant. Neighbour-joining analysis using the three substitution models on the combined data yielded trees with similar topology and bootstrap values. Parsimony analysis of the alignment yielded 108 most parsimonious trees, one of which is shown in Fig. 1. Between the neighbour-joining and parsimony analyses, the trees differed in the hierarchical order of the main families (data not shown). The phylogenetic trees obtained from the large subunit sequences presented a basal polytomy with no bootstrap support for any hierarchical clustering. However, isolates of all of the families included in the analysis grouped together, for example isolates of the *Schizoparme* complex (99 % bootstrap support), the *Cryphonectria/Endothia* complex (78 % bootstrap support), the *Valsaceae* (94 % bootstrap support) and the *Gnomoniaceae* and *Melanconidaceae* (100 % bootstrap support). Irrespective of what analysis method was used, sequences of the *Harknessia* isolates remained at a basal polytomy, with *Dwiroopa lythri* (D.F. Farr & Rossman) D.F. Farr & Rossman always forming a sister taxon and the two isolates of *Harknessia insueta* B. Sutton forming a distinct and more distant cluster.

For ITS, calmodulin and β-tubulin approximately 640, 500 and 870 bases were determined, respectively, for the isolates as indicated in Table 1. The manually adjusted alignment consisting of all three loci contained 34 taxa (including the two outgroups) and 2100 characters including alignment gaps (TreeBASE accession S1149). The combined data set used for phylogenetic analysis contained 1795 characters, of which 742 were parsimony-informative, 264 were variable and parsimony-uninformative, and 789 were constant. The topology of the trees generated with neighbour-joining analysis with the uncorrected "p" and Kimura-2-parameter models were identical, whereas the Jukes-Cantor model yielded a tree that differed from the other two models in the order of the clades in the higher hierarchy (data not shown). Other trees obtained using distance analysis on each individual dataset are also deposited in TreeBASE. Parsimony analysis of the combined data yielded 96 most parsimonious trees, one of which is shown in Fig. 2. Between the neighbour-joining and parsimony analyses, the trees differed only in the order of taxa and groupings within the main clades (data not shown). Three species, namely *H. renispora* H.J. Swart, *H. syzygii* Crous, M.J. Wingf. & Nag Raj and the two strains of *H. eucalyptorum* Crous, M.J. Wingf. & Nag Raj did not form a close association within any of the two main clades. The remaining isolates were divided into two main clades, the first clade having a bootstrap support value of 100 % and containing isolates of *H. fusiformis* Crous, M.J. Wingf. & Nag Raj and *H.* "*uromycoides*" (Speg.) Speg. as well as a well-supported group (100 % bootstrap support) of *H. weresubiae* Nag Raj, DiCosmo & W.B. Kendr. sequences. The second main clade has a bootstrap support value of 71 % and contained a single sequence of *H. karwarrae* B. Sutton & Pascoe, and a clade (100 % bootstrap support) containing isolates of *H. globispora* sp. nov., *H. eucalypti* Cooke and *W. molokaiensis* Crous & J.D. Rogers as well as groups of sequences of *H. protearum* sp. nov. (100 % bootstrap support), *H. leucospermi* Crous & Viljoen (basal polytomy), *H. capensis* sp. nov. (99 % bootstrap support) and *H. hawaiiensis* F. Stevens & E. Young (100 % bootstrap support).

Taxonomy

The present study has resulted in the recollection of several *Harknessia* species that have lacked any cultures to date, and has also added valuable information pertaining to their host range and distribution. Three species are newly described, two from *Proteaceae*, and another from *Myrtaceae*. *Harknessia insueta* was also collected from Brazil and Colombia, and based on molecular data, is relocated to a new genus. The allocation of *H. lythri* to *Dwiroopa* (Farr & Rossman 2003) is also supported by its distinct macro- and meso-conidia.

Apoharknessia Crous & S. Lee, **gen nov.**
MycoBank MB500065.

Etymology: In reference to the presence of apical apiculus and similarity to *Harknessia*.

Genus *Harknessiae* simile sed conidiis sursum apiculatis distinguendum.

Similar to *Harknessia*, but distinct in having a hyaline, apical apiculus, and not forming fluffy aerial mycelium on oatmeal or malt extract agar, but growing within the medium, and also sporulating on naked hyphae.

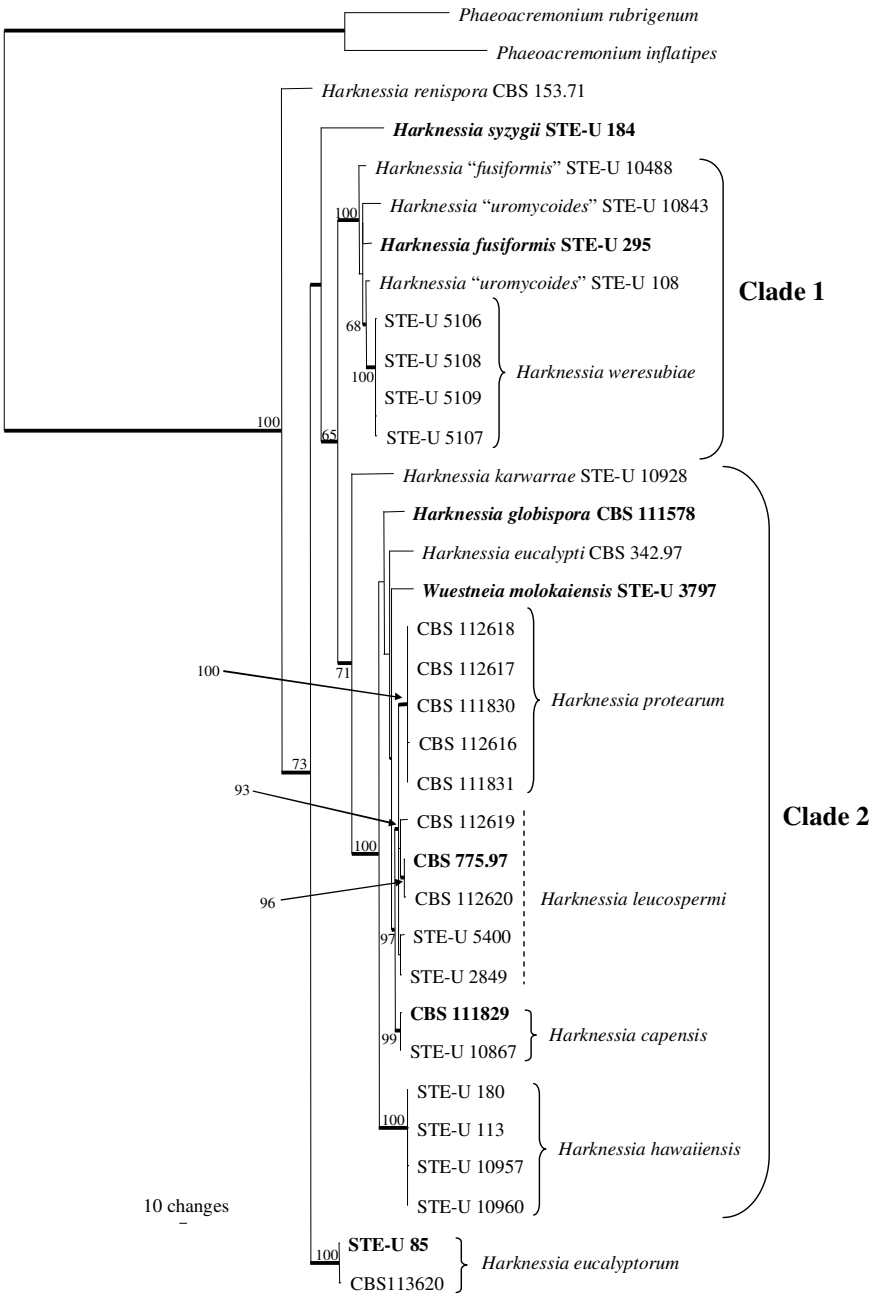


Fig. 2. One of 96 most parsimonious trees obtained from a combined analysis of ITS, calmodulin and β-tubulin sequence data. (TL = 1576 steps, CI = 0.867, RI = 0.894, RC = 0.775). The numbers at the nodes represent bootstrap support values based on 1000 resamplings (values of 65 % and higher are shown). Branches that appear in the strict consensus tree are indicated by thickened lines. Type and ex-type isolates are indicated in bold. The bar indicates 10 changes. The tree was rooted to *Phaeoacremonium rubrigenum* (GenBank accessions AF197988 / AY579288 / AF246802) and *Phaeoacremonium inflatipes* (GenBank accessions AF197990 / AY579290 / AF246805).

Type species: *Apoharknessia insueta* (B. Sutton) Crous & S. Lee, comb. nov.

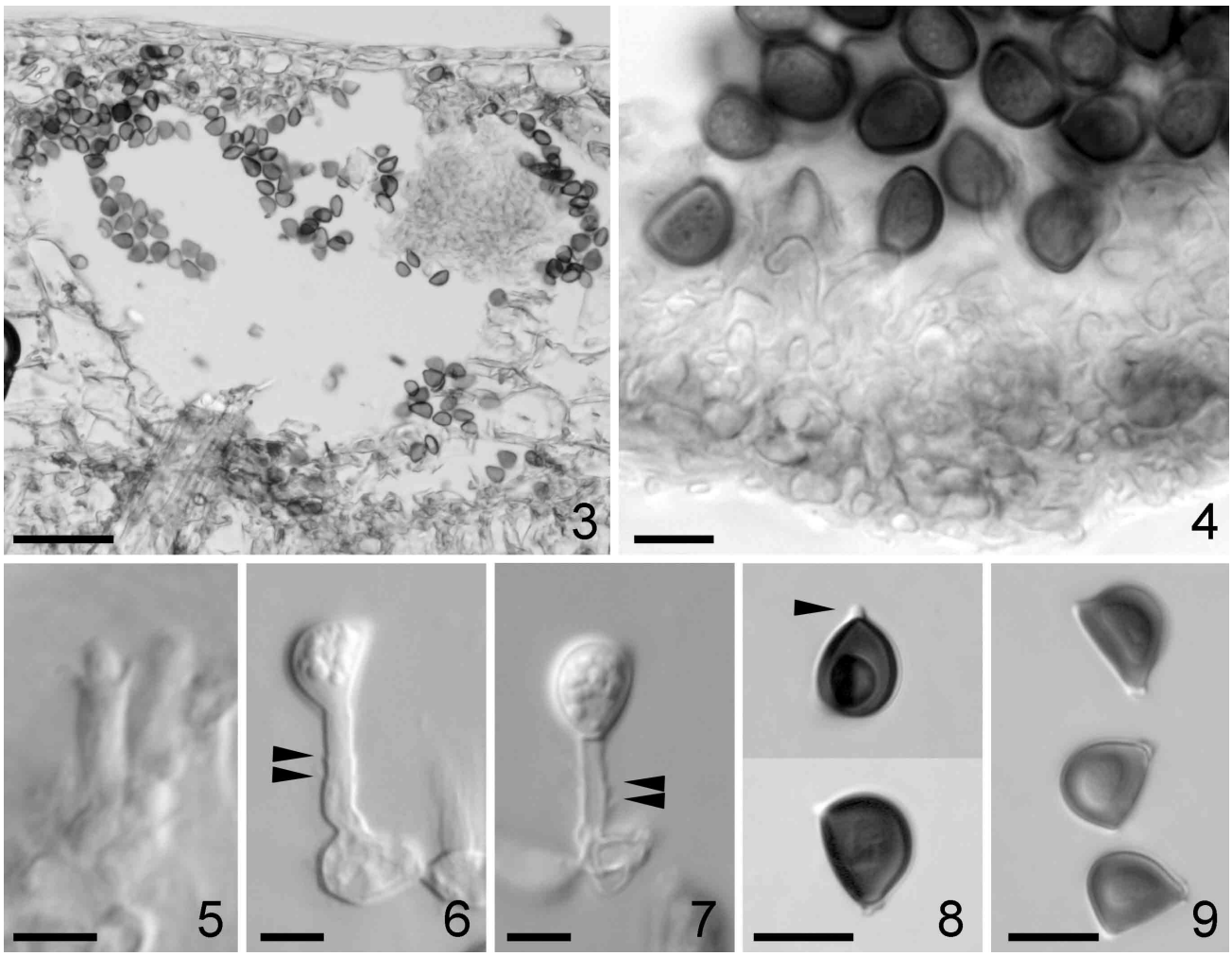
Apoharknessia insueta (B. Sutton) Crous & S. Lee, **comb. nov.** MycoBank MB500066. Figs 3–10.

≡ *Harknessia insueta* B. Sutton, Mycol. Pap. 123: 20. 1971.

See Sutton (1980) and Nag Rag (1993) for descriptions and additional illustrations.

Specimens examined: **Mauritius**, Les Urares, on leaves of *Eucalyptus robusta*, 19 Jan. 1933, G. Orian, IMI 22697 (**holotype**). **Brazil**, Amazonia, Jari, on leaves of *Eucalyptus pellita*, 8 Jul. 1996, P.W. Crous, herb. CBS 9913, (**epitype, designated here**), culture ex-type CBS 111377 = STE-U 1451. **Colombia**, Suiza, leaf spots on *Eucalyptus* sp., Jan. 2004, M.J. Wingfield, CBS 114575 = STE-U 10947.

Notes: Of the synonyms listed for *Harknessia* by Nag Raj (1993), *Mastigonetron* Kleb. was erected for *M. fuscum* Kleb. This genus is a potential home for *H. insueta*, as *M. fuscum* (≡ *H. fusca* (Kleb.) Nag Raj & DiCosmo) has setulose apical appendages. However,



Figs 3–9. *Apoharknessia insueta* (CBS 111377). 3. Section of conidioma formed in banana leaf on CMA. 4. Peridium. 5–7. Conidiogenous cells showing percurrent proliferation (arrowheads). 8, 9. Conidia with hyaline, apical apiculus (arrowhead). Scale bars: 3 = 50 µm; 4–9 = 10 µm.

this species has a *Wuestneia* teleomorph, *W. fusca* (Nag Raj & DiCosmo) J. Reid & C. Booth, and it is thus unlikely that it will cluster separate from *Harknessia*. For this reason, we chose to erect a new genus to accommodate *H. insueta*. Pending further collections and molecular analysis, it will become clear whether other species of *Harknessia* need to also be accommodated here.

In culture *A. insueta* is distinct from species of *Harknessia* in that it does not form fluffy aerial mycelium, but colonizes the agar by growing within the medium. Furthermore, it sporulates within a week, thus much sooner than species of *Harknessia*. Conidia are initially seen forming directly on hyphae in *Apoharknessia*, contrasting with *Harknessia*, which tends to form conidiomata from the onset.

Dwiroopa lythri (D.F. Farr & Rossman) D.F. Farr & Rossman, Mycoscience 44: 445. 2003. Figs 11–15.

≡ *Harknessia lythri* D.F. Farr & Rossman, Mycologia 93: 997. 2001.

See Farr & Rossman (2001) for description and additional illustrations.

Specimen examined: **U.S.A.**, St. Paul, on *Lythrum salicaria*, 1996, E.J. Katovich, BPI 747560 (**holotype**), culture ex-type CBS 109755.

Notes: *Dwiroopa lythri* is distinguished from *Harknessia* by having prominent longitudinal slits in the conidia, which are different in nature from the longitudinal bands of lighter pigment observed in species of *Harknessia* (Nag Raj 1993, Farr & Rossman 2003). Its macroconidia are further distinguished by having very thick walls (up to 2 µm thick), unlike the thin-walled conidia of *Harknessia* species. Three conidial types have been reported for species of *Dwiroopa*, namely macroconidia, mesoconidia and phialoconidia (presumably spermatia) (Farr & Rossman 2003). Cultures of *D. lythri* were observed to form macroconidia and mesoconidia on oatmeal agar (Gams *et al*. 1998). Mesoconidia, which have not formerly been observed for this species, were pale brown, ellipsoidal, with rounded apices and truncate bases, frequently with a marginal frill, 6–7 × 5–6 µm.

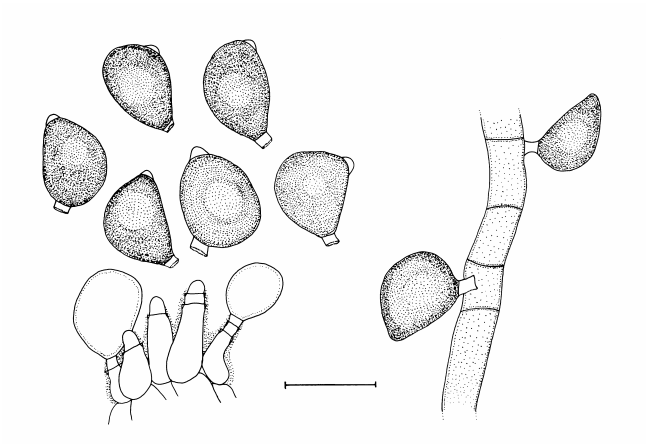
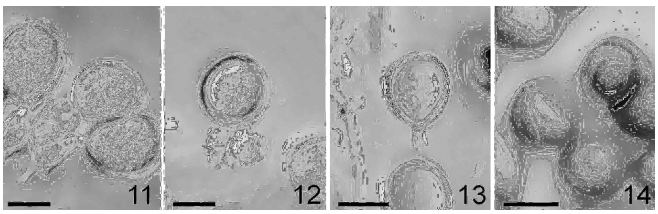


Fig. 10. Conidia of *Apoharknessia insueta* formed in culture in conidiomata (left) and on hyphae (right) (CBS 111377). Scale bar = 10 µm.



Figs 11–14. *Dwiroopa lythri* (CBS 109755). 11, 12. Conidia with conidiogenous cells. 13. Conidium with basal appendage. 14. Conidia with prominent longitudinal slits. Scale bars = 10 µm.

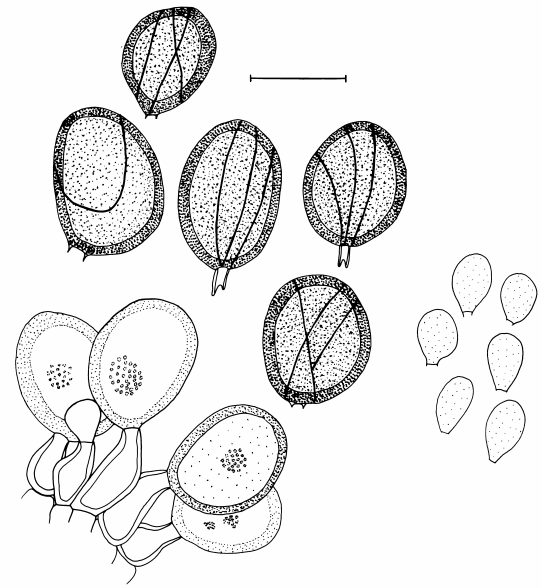


Fig. 15. Macro- and microconidia of *Dwiroopa lythri* formed in culture (CBS 109755). Scale bar = 10 µm.

A major difference between *Harknessia* and *Dwiroopa* lies in its conidiogenesis. Conidiogenous cells of *Dwiroopa* are thick-walled, and the point of conidial attachment forms a slightly darker scar upon dehiscence, with a minute marginal frill. Occasionally the remains of the conidiogenous cell are visible as a minute basal appendage, but this is different from *Harknessia*, in that the appendages appear shattered and thick-walled, thus being more of a marginal frill, and unlike the appendages in *Harknessia* which are longer, tubular, and thin-walled. *Dwiroopa* tends to form multilocular conidiomata in culture, and has three conidial types (Farr & Rossman 2003), which is not the case in *Harknessia*.

Harknessia capensis S. Lee & Crous, **sp. nov.** MycoBank MB500067. Figs 16–25.

Etymology: In reference to the Western Cape Province, where this fungus was collected.

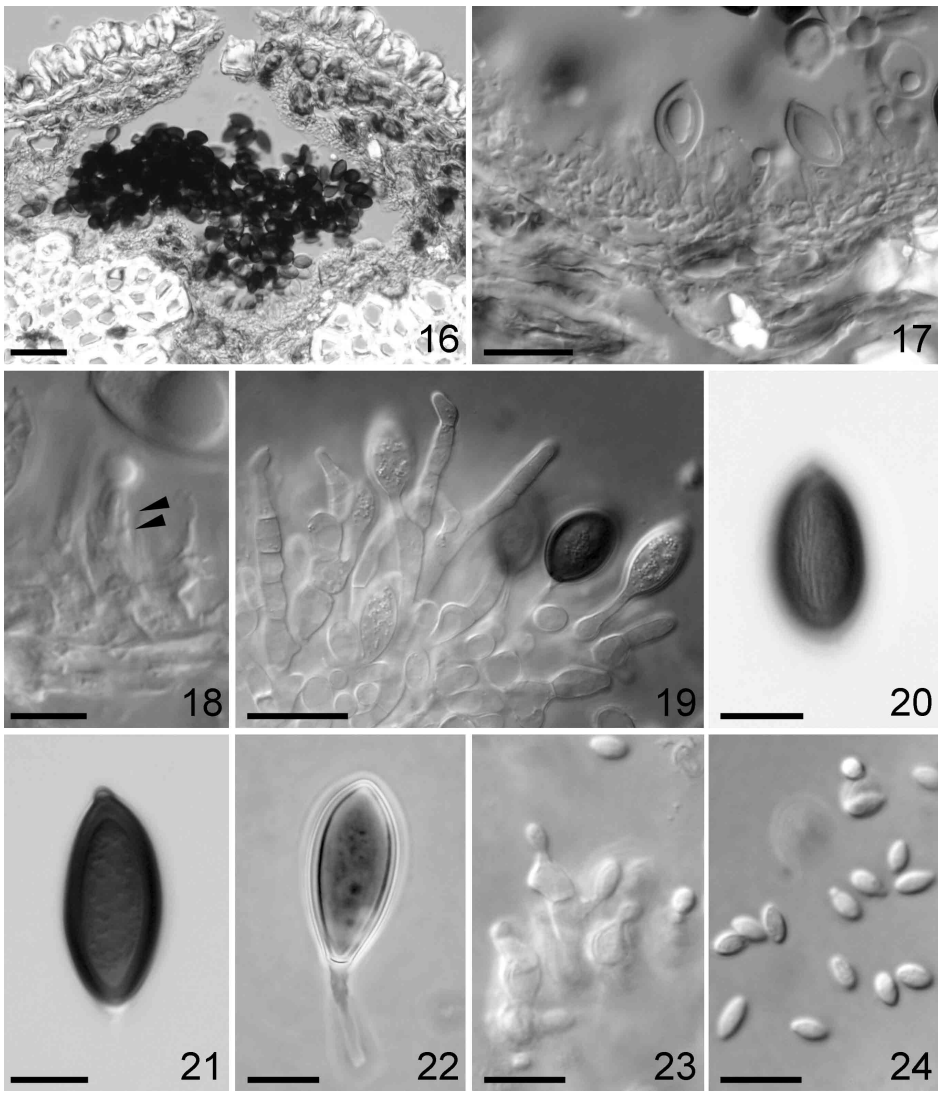
Harknessiae eucryptae et *H. eucalypti* similis, sed conidiis majoribus (18–)21–23(–26) × (9–)11(–12) µm (in medio 22.2 × 10.9 µm) distinguenda.

Caulicolous and foliicolous. *Conidiomata* separate to gregarious, subepidermal, unilocular, conical, up to 380 µm in width, and up to 300 µm in height. *Peridium* pseudoparenchymatous, 10–12.5 µm wide at the base and the sides, up to 20 µm wide around the apex, composed of 2–3 layers of pale yellow to hyaline cells at the base and the sides, or up to 7 layers at the apex. *Conidiophores* reduced to conidiogenous cells lining the base of conidiomatal cavity. *Conidiogenous cells* 7.5–8.5 × 4–6 µm, lageniform to ampulliform, hyaline, smooth, percurrently proliferating once or twice. *Conidia* (18–)21–23(–26) × (9–)11(–12) µm (av. 22.2 × 10.9 µm) *in vivo*, (15–)17–19(–22) × (9–)10–11(–12) µm (av. 18 × 10.5 µm) *in vitro*, ventricose or oval to ellipsoidal with an apiculus and truncate base, pale brown with a paler area at the apiculus, smooth, except for restricted areas bearing longitudinal striations which are prominent on most conidia, surrounded by a thin, persistent mucous sheath when mounted in water. *Basal appendage* 1–3(–10) × 1.5–3 µm *in vivo*, 5–22 × 2–3 µm *in vitro*, hyaline, tubular, smooth, thin-walled, flexuous. *Microconidiogenous cells* developing *in vitro*, forming in the same conidiomata that produce macroconidia, subcylindrical or ampulliform, hyaline, with apical periclinal thickening, 4–10 x 3–5 µm. *Microconidia* hyaline, smooth, aseptate, ellipsoid or fusoid, apex obtuse, base truncate, 4–8 × 2–3 µm.

Geographic distribution: South Africa.

Hosts: *Brabejum stellatifolium* L. (*Proteaceae*), *Eucalyptus* sp. (*Myrtaceae*).

Specimens examined: **South Africa**, Western Cape province, Jonkershoek Nature Reserve, on dead twigs and leaf litter of *Brabejum stellatifolium*, 1 Dec. 2000, S. Lee, PREM 58129 (**holotype**); culture ex-type CBS 111829 = STE-U 5468; Stellenbosch Mountain, on *Eucalyptus* leaves, Nov. 2003, P.W. Crous, herb. CBS 9880, culture CBS 115061 = STE-U 10867.



Figs 16–24. *Harknessia capensis* (PREM 58129; Figs 16–18, 20, 21 *in vivo*; Figs 19, 22–24 *in vitro*) 16. Section through condioma. 17. Peridium. 18. Conidiogenous cell showing percurrent proliferation (arrowheads). 19. Condiogenous cells formed in culture. 20–22. Conidia with striations, apical apiculus and basal appendage. 23. Microconidiogenous cells and microconidia. 24. Microconidia. Scale bars: 16 = 50 µm; 17–19 = 20 µm; 20–24 = 10 µm.

Cultural characteristics: Colonies sterile on MEA, but sporulating sparsely on oatmeal agar, 58 mm diam on MEA after 5 d at 25°C in the dark, circular with entire margins, white, reverse the same. Mycelium woolly, aerial, medium sparse.

Notes: *Harknessia capensis* is morphologically close to *H. eucrypta* (Cooke & Massee) Nag Raj & DiCosmo on *Knightia excelsa* (*Proteaceae*) from New Zealand, but differs in the dimensions of basal appendages and the presence of longitudinal striations in restricted areas of the conidia. Basal appendages of *H. eucrypta* (6–22 × 2–3 µm) are almost twice as long as those of *H. capensis* (Nag Raj & DiCosmo 1981). Conidia of *H. capensis* closely resemble those of *H. eucalypti* on *Eucalyptus* (*Myrtaceae*) in shape and length, but are narrower, (9–)11(–12) µm, than those of *H. eucalypti*, (11–)13–15 µm (Nag Raj 1993).

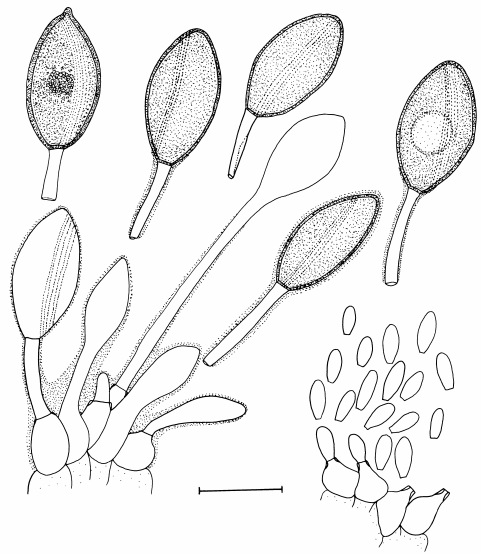
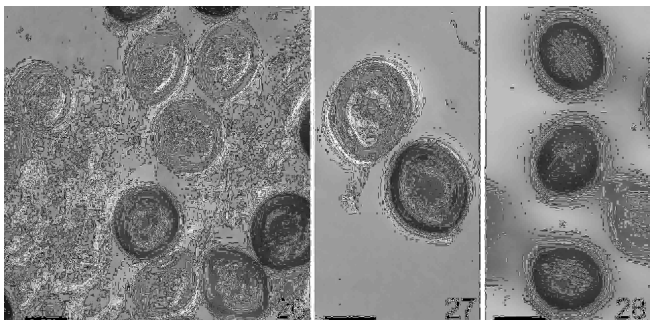


Fig. 25. Macro- and microconidia of *Harknessia capensis* formed in culture (CBS 111829 = STE-U 5468). Scale bar = 10 µm.



Figs 26–28. *Harknessia globispora* (CBS 111578). 26. Young and mature conidia attached to conidiogenous cells. 27, 28. Conidia with basal appendage and striations. Scale bars = 10 µm.

In culture conidia of the holotype collection (*Brabejum*, *Proteaceae*) are (15–)17–19(–22) × (9–)10–11 (–12) µm (av. 18 × 10.5 µm), while those of CBS 115061 (*Eucalyptus*, *Myrtaceae*) are similar, namely (17–)18–20(–21) × (10–)11–12(–13) µm (av. 18.5 × 10.5 µm). In both collections the conidia are striate, and morphologically similar, except that the collection from *Brabejum* has conidia with apices which are sharply rounded, while the *Eucalyptus* strain tends to have conidia with a well-defined apiculus. On DNA sequence, however, we were unable to distinguish these two strains as different species.

Harknessia globispora Crous & S. Lee, **sp. nov.** MycoBank MB500068. Figs 26–29.

Etymology: In reference to the shape of conidia, globose.

Harknessiae globosae similis, sed conidiis (14–)16–19(–20) × (14–)15(–16) µm (in medio 17 × 15 µm) distinguenda.

Leaf spots elongated, situated along the leaf margins, up to 10 mm wide, pale brown, with a red-brown margin. *Conidiomata* separate, scattered, pycnidial, unilocular, subepidermal, globose to subglobose, up to 400 µm diam. *Peridium* pseudoparenchymatous, 10–20 µm thick, composed of 3–4 layers of medium brown cells, textura angularis. *Conidiophores* reduced to conidiogenous cells lining the conidiomatal cavity. *Conidiogenous cells* 6–10 × 4–6 µm, ampulliform to lageniform, hyaline, smooth, proliferating once or twice percurrently, covered in mucus. *Conidia* (14–)16–19(–20) × (14–)15(–16) µm (av. 17 × 15 µm) *in vivo*, brown, with a paler central area consisting of aggregated droplets, globose, covered in a persistent mucous sheath, smooth, except for restricted areas bearing longitudinal striations, base truncate. Basal appendages 2–6 × 2–3 µm, hyaline, tubular, smooth, thin-walled, flexuous. *Microconidia* not seen.

Geographic distribution: Portugal.

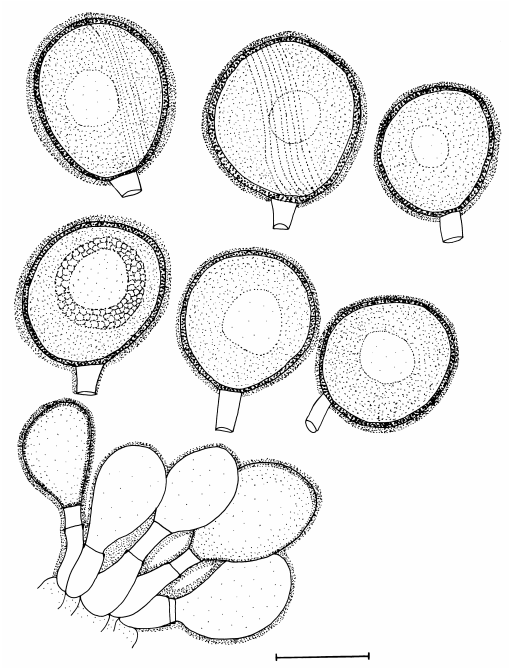


Fig. 29. Conidia of *Harknessia globispora* (herb. CBS 9912). Scale bar = 10 µm.

Host: *Eucalyptus* sp. (*Myrtaceae*).

Specimen examined: **Portugal**, on leaf litter of *Eucalyptus globulus*, Apr. 2000, S. Denman, herb. CBS 9912 (**holotype**); culture ex-type CBS 111578 = STE-U 3711, 3710.

Cultural characteristics: Colonies sporulating sparsely on MEA, 70 mm diam on MEA after 5 d at 25 °C in the dark, circular with entire margins, white, reverse the same, with woolly aerial mycelium.

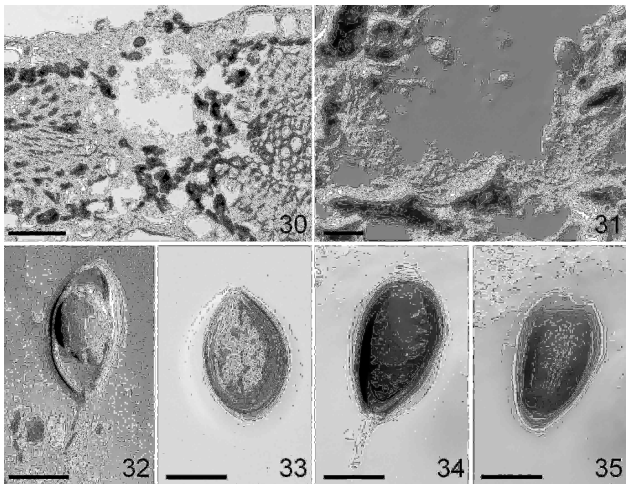
Notes: *Harknessia globispora* has globose conidia similar to those of *H. globosa* B. Sutton, and persistent mucous sheaths as in *H. victoriae* B. Sutton & Pascoe. It differs from both species, however, in having broader conidia, and shorter appendages.

Harknessia protearum Crous & S. Lee, **sp. nov.** MycoBank MB500069. Figs 30–35.

Etymology: In reference to the family of host plants, *Proteaceae*.

Harknessiae eucalypti similis, sed conidiis magis hebeter apiculatis distinguenda.

Caulicolous and foliicolous. *Conidiomata* separate to gregarious, subepidermal, unilocular, globose to subglobose, up to 185 µm wide, up to 160 µm heigh. *Peridium* psueoparenchymatous, 10–12.5 µm thick, composed of 2–3 layers of pale yellow to hyaline cells. *Conidiophores* lining the conidiomatal cavity, reduced to conidiogenous cells.



Figs 30–35. *Harknessia protearum* (PREM 58131). 30. Section through conidioma. 31. Peridium. 32. Conidiogenous cell giving rise to conidium. 33. Young conidium. 34, 35. Conidia with basal appendage and striations. Scale bars: 30 = 100 µm; 31 = 20 µm; 32–35 = 10 µm.

Conidiogenous cells 5–15 × 3–7 µm, lageniform to ampuliform, hyaline, smooth, formed from the inner layer of the conidiomatal wall, proliferating percurrently several times.*Conidia* (21–)23–24(–26) × (12–)13(–14) µm (av. 23.6 × 13 µm), brown, with a paler area near the apex, ventricose or oval to ellipsoidal, smooth, except for restricted areas bearing longitudinal striations, apex bluntly apiculate, base truncate. *Basal appendages* 6–19 × 2–3 µm, hyaline, tubular, smooth, thin-walled, flexuous.

Specimens examined: **South Africa**, Western Cape province, Betty's bay, on leaf litter of *Leucospermum* sp., 26 Jun. 2000, S. Lee, PREM 58130, culture CBS 112617 = STE-U 5406; Kleinmond Nature Reserve, on leaf litter of *Leucospermum oleaefolium*, 11 Jul. 2000, S. Lee, PREM 58131 (**holotype**); culture ex-type CBS 112618 = STE-U 5405; Kleinmond, on dead twig of *Leucadendron* sp., 11 Jul. 2000, S. Lee, PREM 58132, culture CBS 112616 = STE-U 5407; on dead twigs of *Leucospermum* sp., 11 Jul. 2000, S. Lee, PREM 58133, culture CBS 111830 = STE-U 5469; Kogelberg Nature Reserve, on dead twigs of *Leucadendron conocarpodendron*, 3 Nov. 2000, S. Lee, PREM 58134, culture CBS 111831 = STE-U 5470.

Geographic distribution: South Africa.

Hosts: *Leucadendron conocarpodendron* Linn., *Leucadendron* sp., *Leucospermum oleaefolium* R. Br., *Leucospermum* sp. (*Proteaceae*).

Cultural characteristics: Colonies sterile on MEA, 58–66 mm diam on MEA after 5 d at 25 °C in the dark, circular with entire margins, white or white with orange centre (13b), reverse the same. Mycelium woolly, aerial, medium sparse becoming sparser outward.

Notes: *Harknessia protearum* is morphologically similar to *H. eucalypti*, but can be distinguished by its conidial taper. In *H. eucalypti* conidia are more sharply apiculate than those of *H. protearum*. Although *H. eucalypti* has previously been recorded on members of *Proteaceae* from Australia by Sutton & Pascoe (1989), it is possible that this was also *H. protearum*.

Other *Harknessia* spp. studied:

Harknessia eucalypti Cooke, Grevillea 9: 85. 1881. Figs 36–38, 52.
Teleomorph: *Wuestneia epispora* Z.Q. Yuan, Mycol. Res. 101: 195. 1997.

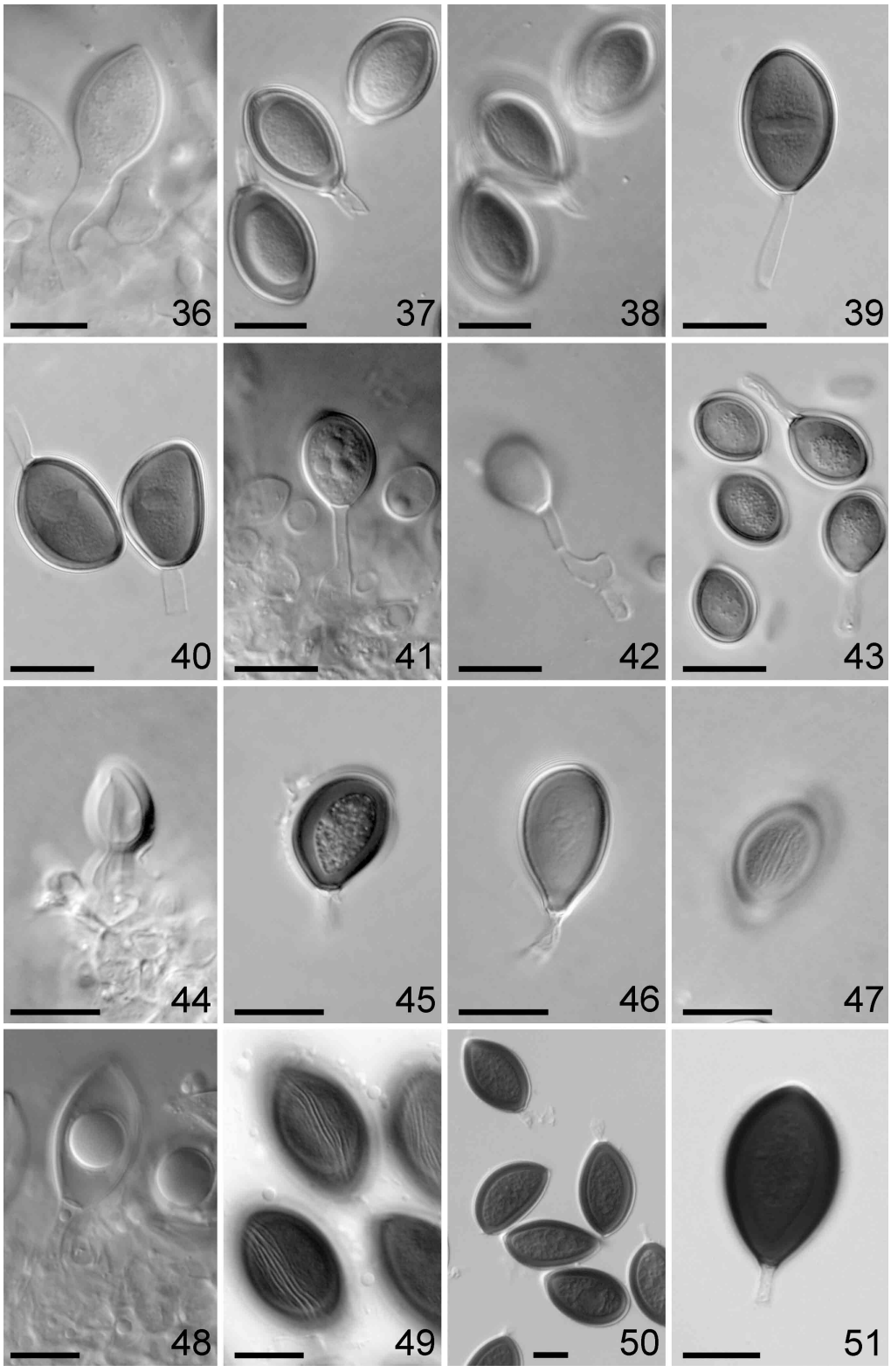
Conidia (11–)18–20(–22) × (11–)12–13(–15) µm (av. 19 × 12 µm), ellipsoid to ovoid with a truncate base and an obtuse to bluntly apiculate apex, smooth, medium brown, with longitudinal striations along the whole length on some conidia; immature conidia covered in a mucous sheath that disappears at maturity. *Basal appendages* 4–10(–20) × 2.5–4 µm, hyaline, tubular, smooth, thin-walled, often collapsing.

Specimens examined: **U.S.A.**, California, Golden Gate Park, on *Eucalyptus* sp., May 1880, H.W. Harkness, IMI 146779 (**holotype**). **Australia**, Tasmania, Westfield, on leaves of *Eucalyptus regnans*, 13 Jul. 1995, Z.Q. Yuan, VPRI 20791, culture CBS 342.97.

Notes: When Yuan & Mohammed (1997) named *Wuestneia epispora*, they concluded that its *Harknessia* anamorph was similar to or identical with *H. eucalypti*. In culture, conidia of *W. epispora* are shorter (11–22 × 11–15 µm) than those of *H. eucalypti* (19–28 × 11–15 µm) on host tissue (Crous *et al.* 1993). However, on host tissue Yuan & Mohammed (1997) recorded conidia to be 18–35 × 12–16 µm (av. 25.4 × 13.9 µm), thus closely matching the conidial averages of *H. eucalypti* (av. 23 × 14 µm) (Nag Raj 1993). We therefore accept this culture as authentic for *H. eucalypti*. An epitype should be designated, however, from fresh material that needs to be collected in San Francisco, California.

Harknessia eucalyptorum Crous, M.J. Wingf. & Nag Raj, Mycologia 85: 109. 1993. Figs 39, 40.
Teleomorph: *Wuestneia eucalyptorum* Crous, M.J. Wingf. & Nag Raj, Mycologia 85: 112. 1993.

Conidia (16–)18–25(–28) × (9–)11(–15) µm (av. 22 × 11 µm), brown, with a paler central area, broadly ventricose, smooth, non-striate, apex obtuse to bluntly apiculate, base truncate. *Basal appendages* 2–11 × 2–3 µm, hyaline, tubular, smooth, thin-walled, flexuous.



Figs 36–51. *Harknessia* spp. 36–38. *Harknessia* anamorph (presumed to be *H. eucalypti*), of *Wuestneia epispora* (CBS 342.97). 39, 40. *Harknessia eucalyptorum* (CBS 111115 = STE-U 85). 41–43. *Harknessia hawaiiensis* (CBS 114811). 44–47. *Harknessia kawarrae* (CBS 115648 = STE-U 10928). 48–51. *Harknessia leucospermi* (PREM 58140). 36, 41, 42, 44, 48. Conidiogenous cells. 37, 39, 40, 43, 45, 46, 50, 51. Conidia with basal appendages. 38, 47, 49. Conidia with striations. Scale bars = 10 µm.

Specimens examined: **South Africa**, Western Cape province, on leaves of *Eucalyptus andrewsii*, 20 Dec. 1989, P.W. Crous, PREM 50830 (**holotype**); culture ex-type CBS 111115 = STE-U 85. **Spain**, Toledo, on leaves of *Eucalyptus* sp., May 2003, P.W. Crous & G. Bills, herb. CBS 9877, culture CBS 113620.

Notes: The Spanish collection closely matches the characteristics of the ex-type strain in morphology.

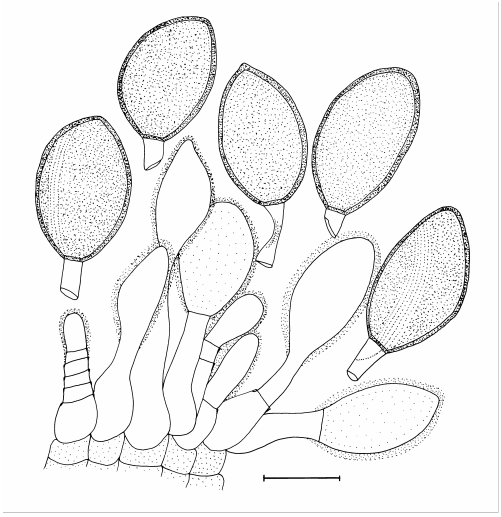


Fig. 52. Conidia of the *Harknessia* anamorph (presumed to be *H. eucalypti*), of *Wuestneia epispora* formed in culture (CBS 342.97). Scale bar = 10 µm.

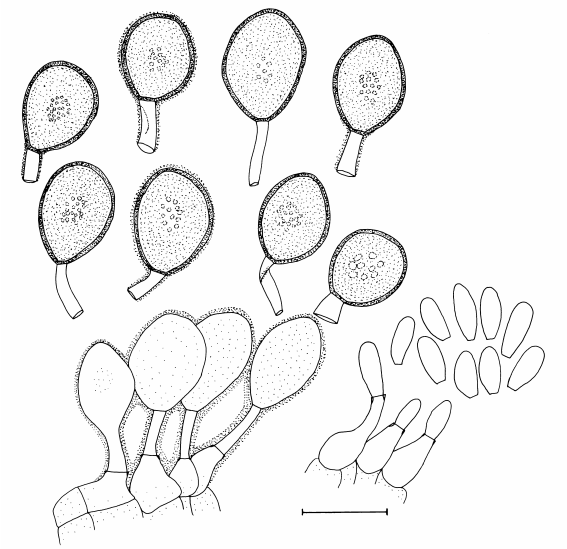


Fig. 53. Macro- and microconidia of *Harknessia hawaiiensis* formed in culture (CBS 114811). Scale bar = 10 µm.

Harknessia fusiformis Crous, M.J. Wingf. & Nag Raj, Mycologia 85: 114. 1993.

Conidia (25–)27–30(–35) × (7–)8–9(–10) µm (av. 29 × 9 µm), brown, with a paler central guttule, frequently with a longitudinal band of lighter pigment, ventricose to fusiform–ellipsoidal, smooth, non-striate, apex obtuse to apiculate, base truncate. *Basal appendages* 45–100 × 2–3 µm, hyaline, tubular, smooth, thin-walled, flexuous.

Specimens examined. **South Africa**, Orange Free State, Bloemfontein, on leaf litter of *Eucalyptus* sp., Feb. 1990, P.W. Crous, PREM 50836 (**holotype**), ex-type culture CBS 110785 = STE-U 295; Bloemfontein, Bain's Game Lodge, on leaves of *Eucalyptus* sp., Jan. 2003, P.W. Crous, herb. CBS 9906, culture CBS 115649 = STE-U 10488.

Notes: The recent collection corresponds well with the type collection, which was also collected at Bloemfontein in South Africa in 1990. Basal appendages are, however, shorter in this collection than observed in the type. Based on the sequence data generated for the different loci investigated here, there are several base pair differences between the recent collection and the ex-type, suggesting that they may be a closely related, but distinct species.

Harknessia hawaiiensis F. Stevens & E. Young, Bull. Bernice P. Bishop Museum 19: 136. 1925. Figs 41–43, 53.

Conidia (10–)11–12 × (8–)9(–10) µm (av. 11.6 × 9 µm), globose to subglobose, brown, smooth except for restricted areas bearing longitudinal striations. *Basal appendages* 3–6 × 1.5–2.5 µm, hyaline, tubular, smooth, thin-walled, flexous.

Specimens examined: **Colombia**, Suiza, on leaf litter of *Eucalyptus* sp., Jan. 2004, M.J. Wingfield, herb. CBS 9905, culture CBS 114811 = STE-U 10957, CBS 115650 = STE-U 10960. **South Africa**, Western Cape Province, Stellenbosch Mountain, on leaves of *Eucalyptus viminalis*, 9 Dec. 1988, P.W. Crous, culture CBS 110728 = STE-U 113; Mpumalanga, on leaves of *Eucalyptus grandis*, 2 Nov. 1989, P.W. Crous, culture CBS 111122 = STE-U 180. **U.S.A.**, Hawaii, Oahu, Waipai, on leaves of *Eucalyptus robusta*, Lyon, IMI 147757 (**holotype**).

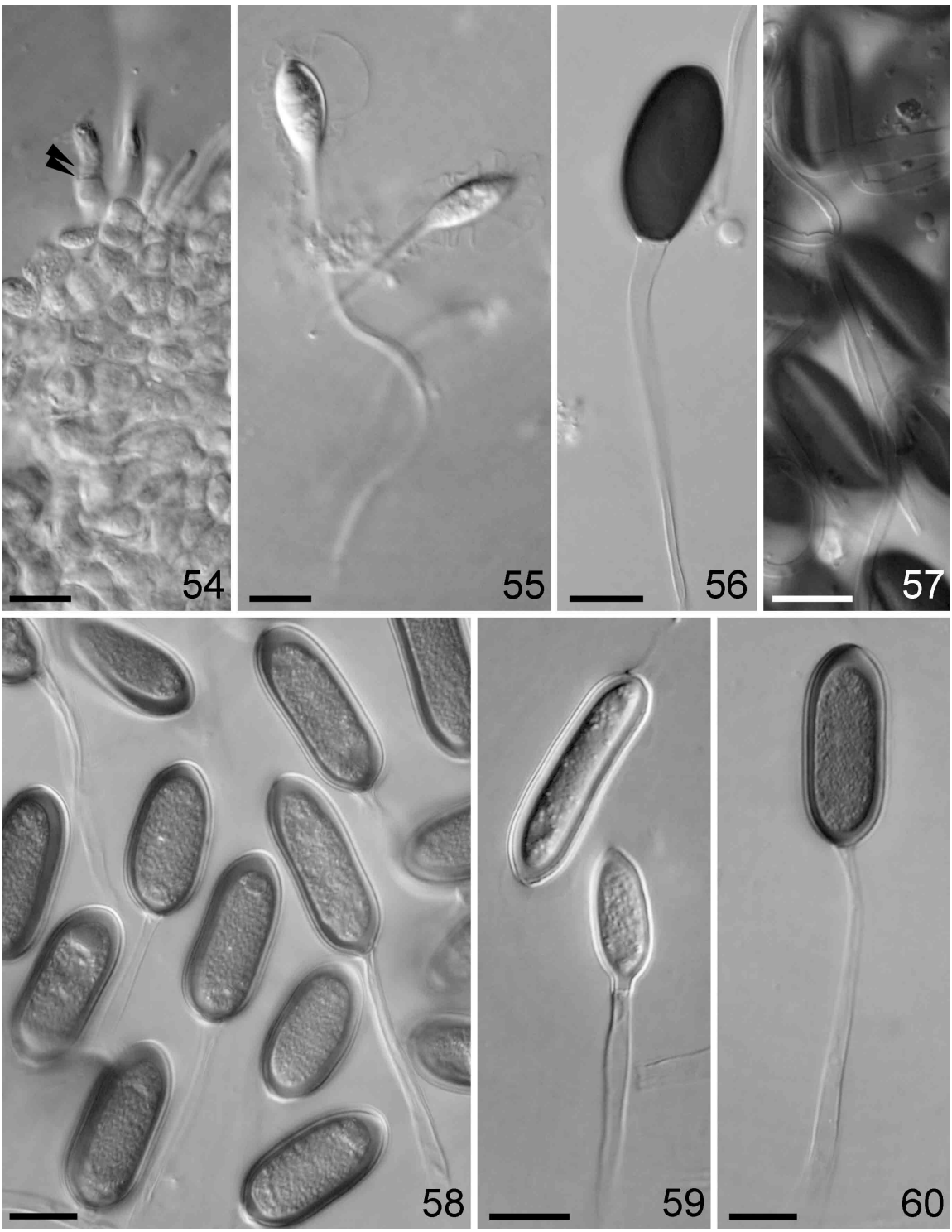
Notes: The longitudinal striations in restricted areas of the conidia, and the presence of a gelatinous sheath has proven to be variable among different isolates of *H. hawaiiensis* (Nag Raj 1993, Crous *et al.* 1993, Yuan *et al.* 2000). Both partial striations and a persistent gelatinous sheath surrounding the conidia were observed among South African isolates. The Colombian strains had conidia which were more ellipsoid to ovate in shape, covered in a thin, persistent mucous sheath (only visible in water mounts). As supported by the DNA phylogeny (Fig. 1), this variation appears to be acceptable within the species.

Harknessia karwarrae B. Sutton & Pascoe, Mycol. Res. 92: 432. 1989. Figs 44–47.

Teleomorph: *Wuestneia karwarrae* (B. Sutton & Pascoe) Z.Q. Zuan, Mycol. Res. 101: 198. 1997.

≡ *Cryptosporella karwarrae* B. Sutton & Pascoe, Mycol. Res. 92: 431. 1989.

Conidia (12–)13–16(–19) × (10–)11(–12) µm (av. 15 × 11 µm), brown, with a paler central guttule, ellipsoid to ventricose, smooth, but sometimes with longitudinal striations along the length of the conidium, apex obtuse to apiculate, base truncate; conidia covered with a persistent mucous sheath, which is clearly visible when mounted in water.



Figs 54–60. *Harknessia* spp. 54–57. *Harknessia uromycoides* (PREM 50843). 54. Conidiogenous cell showing percurrent proliferation (arrowheads). 55. Young conidia covered in mucous sheath. 56. Conidium with basal appendage. 57. Conidia with a longitudinal band of paler pigment. 58–60. Conidia of *Harknessia weresubiae* with long tubular basal appendage (herb. CBS 9903). Scale bars = 10 µm.

Basal appendages 3–8 × 2–3 µm, hyaline, tubular, smooth, thin-walled, flexuous.

Specimen examined: **New Zealand**, North Island, Kerikeri, on leaves of *Eucalyptus botryoides*, 17 Oct. 2003, M. Dick, NZFRI M5098, herb. CBS 9881, culture CBS 115648 = STE-U 10928.

Notes: Pycnidia only developed on leaves after incubation for several days. These were found on green, apparently healthy tissue, suggesting that *H. karwarrae* could be an endophyte of *Eucalyptus*. This collection agrees well with that of the type, which was described from *Acacia glaucoptera* collected in Australia (Sutton & Pascoe 1989). No teleomorph was observed in the present collection.

Harknessia leucospermi Crous & Viljoen, S. Afr. J. Bot. 62: 140. 1998. Figs 48–51.

Conidia (24–)26–27(–30) × (13–)15(–16) (av. 26.5 × 15 µm), ventricose, bluntly apiculate, pale brown, smooth except for restricted areas bearing longitudinal striations. *Basal appendages* 4–15 × 1–3 µm, hyaline, tubular, smooth, thin-walled, flexuous.

Specimens examined: **South Africa**, Western Cape Province, Kirstenbosch National Botanical Garden, leaf litter of *Leucospermum* sp., 20 May 1996, P.W. Crous, PREM 55349 (**holotype**), ex-type culture CBS 775.97 = STE-U 1373; Cape Point National Park, on dead twigs of *Leucospermum praecox*, 23 Feb. 2001, S. Lee, PREM 58135, culture STE-U 5400; Helderberg Nature Reserve, on dead twigs of *Protea laurifolia*, 14 Aug. 2000, S. Lee, PREM 58136, culture CBS 112619 = STE-U 5404; on dead twigs of *Protea burchellii*, 14 Aug. 2000, S. Lee, PREM 58137; on dead twigs and leaves of *Protea* sp., 1 Dec. 2000, S. Lee, PREM 58138; on dead twigs of *Erica mammosa* (*Ericaceae*), 1 Dec. 2000, S. Lee, PREM 58139; on dead twigs of unidentified tree (*Proteaceae*), 1 Dec. 2000, S. Lee, PREM 58140, culture CBS 112620 = STE-U 5403.

Notes: This is the second report of the species from South Africa, and the first report on *Erica mammosa* (*Ericaceae*). The morphology of these specimens matches the description provided by Swart *et al*. (1998), except that conidiomata were described as globose to subglobose, tending to be more conical in shape. Furthermore, in culture conidiomata have even walls, 2–3 layers thick. Given the variation seen in the DNA sequences generated for the different isolates, it appears likely that they represent more than one cryptic species. Morphologically, however, there is significant overlap, and hence we choose to retain these strains in *H. leucospermi* for now.

Harknessia uromycoides (Speg.) Speg., An. Soc. Cient. Argent. 13: 21. 1882. Figs 54–57.

Conidia (20–)22–25(–26) × (10–)11–12(–13) µm (av. 23 × 11.5 µm), brown, with a paler central area, ventricose to ellipsoidal, smooth, non-striate, apex apiculate, base truncate; conidia frequently with a longitudinal band of paler pigment. *Basal appendages* 50–80 × 1.5–3 µm, hyaline, tubular, smooth, thin-walled, flexuous, often collapsing.

Specimens examined: **Argentina**, Buenos Aires, on leaves of *Eucalyptus globulus*, C. Spegazzini, May 1880, IMI 14852, 14853 (**holotype**). **Italy**, Viterbo, Vulci, on *Eucalyptus* leaf litter, Dec. 2003, W. Gams, herb. CBS 9904, culture CBS 115647 = STE-U 10843. **South Africa**, Western Cape Province, Stellenbosch Mountain, on *Eucalyptus* leaf litter, 8 Dec. 1988, P.W. Crous, PREM 50834, culture CBS 110729 = STE-U 108.

Notes: Conidia are slightly wider and shorter than those of *H. spermatoidea*, and do not narrow in the median part. Young, developing conidia tend to be covered in mucus. The present collection corresponds well with the type of *H. uromycoides*, although the basal appendages are shorter than reported for the type from Argentina by Nag Raj (1993) (57–130 × 2–2.5 µm). This species has also been reported from South Africa (Crous *et al*. 1993). Based on the DNA sequence data, it appears that the Italian and South African strains may represent different species within the *H. uromycoides* complex, and thus an epitype strain from Argentina would be required to resolve their status.

Harknessia weresubiae Nag Raj, DiCosmo & W.B. Kendr., Biblioth. Mycol. 80: 53. 1981. Figs 58–60.

Conidia (18–)22–27(–30) × (6–)9–10(–11) µm (av. 25 × 10 µm), olivaceous-brown, with a paler central area, ellipsoidal to subcylindrical, frequently constricted in the central part, smooth, non-striate, apex obtuse, base truncate. *Basal appendages* 22–80 × 2–3 µm, hyaline to pale brown, tubular, smooth, thin-walled, flexuous, collapsing.

Specimens examined: **Australia**, Saddleworth, on *Eucalyptus* leaf litter, 22 Sept. 1979, B. Kendrick, DAOM 173902 (**holotype**). **South Africa**, Western Cape province, Tulbach, on leaf litter of *Eucalyptus* sp., 13 Mar. 2002, P.W. Crous & J. Stone, herb. CBS 9903, cultures CBS 113075 = STE-U 5106, CBS 113074 = STE-U 5107, CBS 113073 = STE-U 5108.

Notes: The species is known from *Eucalyptus* leaf litter collected in Australia, and has now been collected on the same host from South Africa. The morphology of the South African collection closely matches that of the type, but awaits fresh Australian collections before this can be confirmed.

DISCUSSION

The present study has linked cultures to several species of *Harknessia* for which no cultural material had been known so far. In the process, a wider host and geographical range has been established than previously known for many of these taxa. Three new species of *Harknessia* were newly described, while the genus was shown to be heterogeneous, and has subsequently been split to recognize *Dwiroopa* (Farr & Rossman 2003), *Apoharknessia* and *Harknessia*.

Harknessia species known from South Africa

Most of the collections studied here originated from South Africa. Presently there are six species of *Harknessia* known from South Africa (Crous *et al*. 1989, 1993, Swart *et al*. 1998). *Harknessia eucalyptorum*, which is a common species on eucalypts in the Western Cape province, is reported for the first time from eucalypts in Spain. *Harknessia fusiformis* was recollected from its type locality in the Orange Free State, and is presently only known from South Africa, as is *H. syzygii* which occurs on *Syzygium cordatum*. *Harknessia uromycoides* is a relatively well-known taxon with a host range also including non-myrtaceous hosts (Nag Raj 1993). This species is known from eucalypt litter in South Africa (Crous *et al*. 1993), and was also collected on this substrate in Italy in the present study. *Harknessia hawaiiensis* occurs commonly on eucalypts (Crous *et al*. 1993), and was also collected on this host in Colombia. Furthermore, molecular analysis also revealed that the collection with globose conidia assumed by Crous *et al*. (1993) to possibly represent a distinct taxon (PREM 50844, STE-U 113) could, in fact, be accommodated in *H. hawaiiensis*. Several additional collections were also obtained of *H. leucospermi* on other members of the *Proteaceae*, as well as one host in the *Ericaceae*, supporting the fact that it has a wider host range on other hosts in the Fynbos. *Harknessia weresubiae* represents a new record for South Africa, having previously been collected from eucalypt litter in Australia (Nag Raj 1993). *Harknessia protearum*, which is morphologically similar to *H. eucalypti*, is newly described from members of *Proteaceae*. Finally, *H. capensis* was newly described from South Africa on *Brabejum stellatifolium* (*Proteaceae*), and *Eucalyptus* sp. (*Myrtaceae*) making it the ninth species known from this country.

Other *Harknessia* species studied

A further four species were collected from eucalypts in the present study. *Harknessia insueta*, which is known from eucalypts in Brazil, Cuba, Mauritius and the U.S.A. (Sankaran *et al*. 1995), was collected from this host in Brazil and Colombia. A molecular phylogeny indicated this species to cluster apart from *Harknessia* (*H. eucalypti*, type), and hence led us to propose a new genus *Apoharknessia* to accommodate it. *Harknessia globispora* is described as a new species from *Eucalyptus* leaves collected in Portugal. Similarly *H. hawaiiensis* is recorded from *Eucalyptus* leaf litter collected in Colombia. Sutton & Pascoe (1989) described *H. karwarrae* from *Acacia* leaves (*Mimosaceae*) collected in Australia. In the present study it was isolated as presumed endophyte from healthy *Eucalyptus* leaves (*Myrtaceae*) collected in New Zealand, thus once again showing species of *Harknessia* to have wider host ranges than presumed so far.

Generic and familial relationships of *Harknessia*

The genus *Harknessia* as circumscribed by recent revisions (Sutton 1971, 1980, Nag Raj & DiCosmo 1981, Nag Raj 1993), has in the past been heterogeneous. Von Höhnel (1914) removed taxa with hyaline conidia and apical appendages to *Mastigosporella* Höhn., a decision supported by Nag Raj & DiCosmo (1981). Several other genera are listed by Nag Raj as synonyms of *Harknessia*, namely *Caudosporella* Höhn. (based on *H. antarctica* Speg.), *Mastigonetron* Kleb. (based on *M. fuscum* Kleb., which has an apical conidial appendage and a *Wuestneia* teleomorph), and *Cymbothyrium* Petr. (based on *M. sudans* Petr.). Unfortunately, no cultures are presently available to confirm these synonymies.

Teleomorphs of *Harknessia* were presumed to reside in *Cryptosporella* Sacc. (Nag Raj & DiCosmo 1981), although this name was subsequently rejected in favour of *Wuestneia*, which proved to be an older name (Reid & Booth 1989). *Wuestneia* resides in the *Diaporthales*. Von Arx & Müller (1954) erected a separate family, *Cryptosporellaceae*, to accommodate *Cryptosporella*. Due to differences in the ascus apical mechanism, these authors were of the opinion that it fitted better in the broadly delineated *Sphaeriales*, whereas others, including Barr (1978) placed this genus in the *Diaporthales*, a finding confirmed by Castlebury *et al*. (2002) and in the present study.

The placement of *Wuestneia* in the *Melanconidaceae* has been unequivocally recognized by Barr (1990), Hawksworth *et al*. (1995), Eriksson *et al*. (2001), and Samuels & Blackwell (2001). In a preliminary overview of the *Diaporthales* by Castlebury *et al*. (2002), six major lineages in the order were identified based on the LSU nrDNA sequences, of which the *Melanconidaceae* were defined in a restricted sense including the type genus *Melanconis* only, showing close affinity with the *Gnomoniaceae* and excluding *Wuestneia/Harknessia*. A neighbour-joining analysis of the LSU nrDNA sequences of *Harknessia* species produced in this study placed *Wuestneia/Harknessia* as a sister clade of the *Cryphonectria/Endothia* complex, and the *Schizoparme/Pilidiella* complex, which is far apart from the *Melanconidaceae s.str*. In terms of anamorphic features, the *Wuestneia/Harknessia* clade is closer to the members of *Melaconidaceae,* which have holoblastically produced brown, unicellular conidia in stromatic conidiomata (Sutton 1980). These taxa are morphologically distinct from those in the *Cryphonectria/Endothia* complex, which have hyaline, unicellular phialoconidia in multiloculate pycnidia in well-developed stromata. Currently there is no family that accommodates the *Schizoparme/Pilidiella* complex, nor the *Cryphonectria/Endothia* complex. The *Cryptosporellaceae*, as originally erected by von Arx & Müller (1954) for *Cryptosporella xanthostroma*, may provide a home for the *Wuestneia/Harknessia*

complex, provided that *Wuestneia* (= *Cryptosporella*) is the correct teleomorph genus for *Harknessia* anamorphs.

The genus *Wuestneia* is typified by *W. xanthostroma*, which Reid & Booth (1989) found to be associated with a coelomycete anamorph having hyaline conidia. The cultural link, has, however, never been established. If Reid & Booth (1989) are indeed correct, and the hyaline coelomycete is the anamorph of *W. xanthostroma*, then *Wuestneia* is not the correct genus to accommodate *Harknessia* holomorphs. Presently, however, no cultures are available of *W. xanthostroma*, and hence this matter cannot be resolved. The genus *Cryptosporella* is based on *C. hypodermia* (Fr.) Sacc., and this species has a *Disculina* anamorph (Reid & Booth 1989), thus also making *Cryptosporella* (currently *Winterella* O. Kuntze) unavailable for *Harknessia* holomorphs.

The phylogeny of the *Harknessia* species obtained in the present study supported three clades, namely *Harknessia s.str.* (based on *H. eucalypti*), as well as two additional clades, *Apoharknessia* (based on *A. insueta* with apical appendages), and *Dwiroopa* (a genus based on *D. ramya* Subram. & Muthumary, with longitudinal conidial germ slits). In the *Diaporthales*, no family is presently available for the *Schizoparme* complex (*Coniella/Pilidiella* anamorphs), the *Harknessia* complex (*Harknessia/Dwiroopa* anamorphs), the *Cryphonectria* complex (*C. cubensis*), nor *Apoharknessia*. Although morphological observations suggest that the *Schizoparme* complex and the *Harknessia* complex could well be accommodated in the same family, possibly including the *Cryphonectria* complex p.p., further collections would be required to resolve this matter.

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