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 telemorphs accommodated in *Wuestneia*, are shown to represent an undescribed family in the *Diaporthales*, as is *Apoharknessia*, for which no telemorph 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 pycnidial conidiomata, and darkly pigmented conidia with tube-shaped basal appendages, longitudinal striations, and rhexolytic sessession. 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.

Telemorphs of *Harknessia* are known to reside in *Wuestneia* Auerw. 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, 

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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 MgCl2 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 LROR (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.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Cultures</th>
<th>Collection locality</th>
<th>Host plants</th>
<th>ITS</th>
<th>β-tubulin</th>
<th>Calmodulin</th>
<th>LSU</th>
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<td>Lythrum salicaria</td>
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<td>Brachybelopsis stellatfolium</td>
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<td>AY720752</td>
<td>AY720783</td>
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<td>Eucalyptus sp.</td>
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<td>AY720753</td>
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<td>AY720801</td>
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<td>AY720771</td>
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<td>Australia</td>
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<td>W. molokaensis (H. molokaensis)</td>
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<td>USA (Hawaii)</td>
<td>E. robusta</td>
<td>AY720749</td>
<td>AY579335</td>
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</table>

1CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa. 2Ex-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.
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 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.) Spec. as well as a well-supported group (100% bootstrap support) of *H. wersusubiae* 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 *M. molokaiensis* Crous & J.D. Rogers as well as groups of sequences of *H. protearum* sp. nov. (100% bootstrap support), *H. leucospermi* Crous & Viljoen (basal poltomy), *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 *Pseudocercospora* 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.

*Aphoharknessia* Crous & S. Lee, *gen nov.*

MycoBank MB500065.

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

Genus *Harknessia* 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.


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,
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.


**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.
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.

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.

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 × 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.

**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.
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.

_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 high. _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.
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:**


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.


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.


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.


Notes: The Spanish collection closely matches the characteristics of the ex-type strain in morphology.
**PHYLOGENETIC REASSESSMENT OF HARKNESSIA**

**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 hawaiensis* formed in culture (CBS 114811). Scale bar = 10 µm.


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.


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.


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, flexuous.


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. hawaiensis* (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.


Conidia (12–)13–16(–19) × (10–)11(–12) µm (av. 15 × 11 µm), brown, with a paler central guttule, ellipsoidal 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.

**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.


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.


Conidia (20–)22–25(–26) × (10–)11–12(–13) µm (av. 23 × 11.5 µm), brown, with a paler central area, ventricose to elliptoidal, 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.


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. uromyoides, 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. uromyoides complex, and thus an epitype strain from Argentina would be required to resolve their status.


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.


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 collected from its typical locality in the Orange Free State, and is presently only known from South Africa, as is H. syzygii which occurs on Syzygium cordatum. Harknessia urymycoidea 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 hawaiensis 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. hawaiensis. 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 wereusubiae 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. hawaiensis 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 Höhn (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 Cymbothryum Petr. (based on M. sudans Petr.). Unfortunately, no cultures are presently available to confirm these synonyms.

Telemorphs of Harknessia were presumed to reside in Cryptosporella (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 Cryptonectria/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 Melanconidaceae, which have holoblastically produced brown, unicellular conidia in stromatic conidiomata (Sutton 1980). These taxa are morphologically distinct from those in the Cryptonectria/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 Cryptonectria/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* (= *Cryptospora*) 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 *Cryptospora* is based on *C. hypodermia* (Fr.) Sacc., and this species has a *Disculina* anamorph (Reid & Booth 1989), thus also making *Cryptospora* (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, *Apolharknessia* (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/Piliidiella* anamorphs), the *Harknessia* complex (*Harknessia/Dwiroopa* anamorphs), the *Cryphonectria* complex (*C. cubensis*), nor *Apolharknessia*. 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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