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RESEARCH ARTICLE

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A re-appraisal of *Harknessia* (*Diaporthales*), and the introduction of *Harknessiaceae* fam. nov.

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Key words

biodiversity
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Harknessiaceae
ITS
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Abstract *Harknessiaceae* is introduced as a new family in the ascomycete order *Diaporthales* to accommodate species of *Harknessia* with their *Wuestneia*-like teleomorphs. The family is distinguished by having pycnidial conidiomata with brown, furfuraceous margins, brown conidia with hyaline, tube-like basal appendages, longitudinal striations, and rhexolytic secession. Six species occurring on *Eucalyptus* are newly introduced, namely *H. australiensis*, *H. ellipsoidea*, *H. pseudohawaiiensis*, and *H. ravenstreetina* from Australia, *H. kleinzeeina* from South Africa, and *H. viterboensis* from Italy. Epitypes are designated for *H. spermatoidea* and *H. weresubiae*, both also occurring on *Eucalyptus*. Members of *Harknessia* are commonly associated with leaf spots, but also occur as saprobes and endophytes in leaves and twigs of various angiosperm hosts.

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INTRODUCTION

Members of the genus *Harknessia* have a worldwide distribution, and are commonly associated with leaves and branches (twigs) of a wide range of hosts (Nag Raj 1993, Sankaran et al. 1995, Farr & Rossman 2001). Although some species have been reported as being associated with leaf spots (Crous et al. 1989, 1993), many have been isolated from leaf and twig litter (Sutton & Pascoe 1989, Swart et al. 1998, Crous & Rogers 2001, Lee et al. 2004, Marincowitz et al. 2008), or from leaves with symptoms of tip dieback or leaf scorch (Fig. 1). Conidiomata readily develop in moist chambers, and species appear to be endophytic (Bettuci & Saravay 1993), often fruiting on leaf spots of more aggressive foliar pathogens. Although several *Harknessia* species may be pathogenic, not much is known about their pathogenicity, and in general they are regarded of little economic importance (Park et al. 2000). Species of *Harknessia* occur on diverse gymnosperm and dicotyledonous hosts, with the genus *Eucalyptus* (*Myrtaceae*) harbouring up to 21 of the 53 species recognised. Several major treatments have focused on revising the genus (Sutton 1971, 1980, Nag Raj & DiCosmo 1981, Nag Raj 1993), although only a few studies have employed an integrated approach with molecular data to resolve species boundaries and host specificity (Castlebury et al. 2002, Lee et al. 2004, Summerell et al. 2006, Crous et al. 2007).

The genus *Harknessia* is characterised by having stromatic to pycnidial conidiomata, and dark brown conidia with tube-shaped basal appendages, longitudinal striations, and rhexolytic secession. Taxa with hyaline conidia and apical appendages were placed in *Mastigosporella* (von Höhnel 1914), while *Apoharknessia* was introduced for species with brown conidia and apical as well as basal appendages (Lee et al. 2004), and *Dwiroopa* for species with very thick conidial walls and longitudinal slits (Farr & Rossman 2003). Several genera were also seen as synonyms, namely *Caudosporella*, *Mastigonetron*, and *Cymbothyrium* (Nag Raj & DiCosmo 1981).

Teleomorphs of *Harknessia* were initially described in *Cryptosporella* (Nag Raj & DiCosmo 1981) (*Cryptosporellaceae*; von Arx & Müller 1954), which Reid & Booth (1989) reduced to synonymy with the older *Wuestneia* (*Diaporthales*) (Barr 1978, Castlebury et al. 2002, Lee et al. 2004). Seven of the 13 *Wuestneia* species known to date have been linked to *Harknessia* anamorphs (Reid & Booth 1989, Sutton & Pascoe 1989, Crous et al. 1993, Yuan & Mohammed 1997, Crous & Rogers 2001) (Fig. 2, 3).

Castlebury et al. (2002) provided an overview of *Diaporthales*, recognising six major lineages, of which *Melanconidaceae* had an affinity with *Gnomoniaceae*, highlighting unresolved complexes such as the *Wuestneia*/*Harknessia* complex, *Cryphonectria*/*Endothia* complex, and the *Schizoparme*/*Pilidiella* complex. Subsequent studies have resolved the latter two complexes to represent the *Cryphonectriaceae* (Gryzenhout et al. 2006) and *Schizoparmaceae* (Rossman et al. 2007), respectively. The *Wuestneia*/*Harknessia* complex has still remained unresolved within *Diaporthales*. The aims of the present study were to introduce a family for the *Wuestneia*/*Harknessia* complex, and to name several newly collected species.

MATERIALS AND METHODS

Isolates

Symptomatic or dead leaves and twigs were collected in different countries from a wide range of hosts (Table 1). Samples were incubated in damp chambers for 2 – 3 d before examination. Single-spore isolation was carried out and cultures were established on malt extract agar (MEA) as described by Crous et al. (1991). Colonies were subcultured onto 2 % potato-dex-

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Fig. 1 Leaf spot disease symptoms associated with *Harknessia* spp. on different *Eucalyptus* hosts. a. *H. fusiformis* (CPC 13649); b. *H. hawaiiensis* (15003); c, d. *H. rhabdosphaera* (CPC 13593 and CPC 12847); e. *H. globispora* (CPC 14924); f. *H. eucalyptorum* (CPC 12697).

trose agar (PDA), MEA, and oatmeal agar (OA) (Crous et al. 2009b), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. Reference strains are maintained in the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004).

DNA phylogeny

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon.

For species delimitation, ITS was supplemented with the partial gene sequences for calmodulin (CAL), determined using the primers CAL-228F (Carbone & Kohn 1999) and CAL-737R (Carbone & Kohn 1999) or CAL2Rd (Quaedvlieg et al. 2011) and beta-tubulin (TUB), amplified and sequenced using the primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995). Amplification conditions followed Lee et al. (2004). The sequence alignment and subsequent phylogenetic analyses for all the above were carried out using methods described by Crous et al. (2006). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses (see TreeBASE); the remaining gaps were treated as 'fifth state' data. Sequence data were deposited in GenBank (Table 1) and the alignments and trees in TreeBASE (http://www.treebase.org).

Taxonomy

Culture characteristics were determined in triplicate from MEA plates after 1 mo of incubation at 25 °C in the dark, and colours determined according to Rayner (1970). Measurements and photographs were made from structures mounted in clear lactic acid. The 95 % confidence intervals were derived from 30 observations (× 1 000 magnification), with the extremes given in parentheses. Ranges of the dimensions of other characters are given. Observations were made with a Zeiss V20 Discovery stereo microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software.

Fig. 2 *Harknessia eucalyptorum* and its teleomorph (CPC 12697). a. Leaf spot symptoms on *Eucalyptus* sp.; b. ascomatum with short neck, oozing ascospores; c, d. paraphyses and asci; e–i. asci; j. paraphyse and ascal tip; k, l. asci; m. ascospores; n. conidiomata oozing conidia; o–q. conidia with basal appendages and central guttules. — Scale bars = 10 µm.

Table 1 *Harknessia* and *Harknessia*-like isolates included in the morphological and/or phylogenetic analyses.

Species	Culture accession numbers[1,2]	Substrate	Country	Collector	GenBank accession numbers[3]			
					ITS	TUB	CAL	LSU
Apoharknessia insueta	CPC 10947; CBS 114575	Leaf spots on *Eucalyptus* sp.	Colombia	M.J. Wingfield	–	–	–	AY720813
	CPC 11775	*Yucca elephantipes*	Costa Rica	A. Igram	JQ706082	–	–	JQ706209
	CPC 1451; CBS 111377[ET]	Leaves of *Eucalyptus pellita*	Brazil	P.W. Crous	JQ706083	–	–	AY720814
Foliocryphia eucalypti	CPC 12494; CBS 124779[ET]	*Eucalyptus coccifera*	Australia: Tasmania	C. Mohammed	GQ303276	JQ706128	–	GQ303307
Harknessia australiensis	CPC 13596; CBS 132120	Leaves of *Eucalyptus sclerophylla*	Australia: New South Wales	B.A. Summerell	JQ706084	JQ706129	JQ706170	JQ706210
	CPC 15029[ET]; CBS 132119	Leaves of *Eucalyptus dissita*	Australia: New South Wales	B.A. Summerell	JQ706085	JQ706130	JQ706171	JQ706211
Harknessia capensis	CPC 10867; CBS 115061	*Eucalyptus leaves*	South Africa: Western Cape Province	P.W. Crous	AY720718	AY720750	AY720781	AY720815
	CPC 5468; CBS 111829[ET]	Dead twigs and leaf litter of *Brabejum stellatifolium*	South Africa: Western Cape Province	S. Lee	AY720719	AY720751	AY720782	AY720816
Harknessia ellipsoidea	CPC 13077; CBS 132122	Leaves of *Eucalyptus propinque*	Australia: New South Wales	B.A. Summerell	JQ706086	JQ706131	JQ706172	JQ706212
	CPC 17111[ET]; CBS 132121	Leaves of *Eucalyptus* sp.	Australia: Queensland	P.W. Crous & R.G. Shivas	JQ706087	JQ706132	JQ706173	JQ706213
	CPC 17113[ET]	Leaves of *Eucalyptus* sp.	Australia: Queensland	P.W. Crous & R.G. Shivas	JQ706088	JQ706133	JQ706174	JQ706214
Harknessia eucalypti	CBS 342.97	*Eucalyptus regnans*	Australia: Tasmania	Z.-Q. Yuan	AY720745	AY720777	AY720808	AF408363
	CPC 13643	*Eucalyptus regnans*	Australia: Tasmania	B.A. Summerell	JQ706089	JQ706134	JQ706175	JQ706215
Harknessia eucalyptorum	CBS 113620	Leaves of *Eucalyptus* sp.	Spain	P.W. Crous & G. Bills	AY720746	AY720778	AY720809	AY720839
	CPC 85; CBS 111115[ET]	Leaves of *Eucalyptus andrewsii*	South Africa: Western Cape Province	P.W. Crous	AY720747	AY720779	AY720810	AY720840
	CPC 11302	*Eucalyptus* sp.	Italy	W. Gams	JQ706090	JQ706135	JQ706176	–
	CPC 12697	Leaf litter of *Eucalyptus* sp.	South Africa: Western Cape Province	P.W. Crous	JQ706091	JQ706136	JQ706177	JQ706216
	CPC 13074	–	Italy	W. Gams	JQ706092	JQ706137	–	JQ706217
	CPC 14951	*Eucalyptus* sp.	Portugal	P.W. Crous	JQ706093	JQ706138	JQ706178	JQ706218
	CPC 14954	*Eucalyptus* sp.	Portugal	P.W. Crous	JQ706094	–	–	JQ706219
	CPC 19659	*Eucalyptus cypellocarpa*	Australia: Northern Territory	P.W. Crous	JQ706095	–	–	–
Harknessia fusiformis	CPC 295; CBS 110785[ET]	Leaf litter of *Eucalyptus* sp.	South Africa: Orange Free State	P.W. Crous	AY720721	AY720753	AY720784	AY720818
	CPC 10488; CBS 115649	Leaves of *Eucalyptus* sp.	South Africa: Orange Free State	P.W. Crous	AY720720	AY720752	AY720783	AY720817
	CPC 11124	*Eucalyptus* sp.	New Zealand	J. Stalpers	JQ706096	JQ706139	JQ706179	–
	CPC 13649	*Eucalyptus globulus*	Australia: Tasmania	B.A. Summerell	JQ706097	JQ706140	JQ706180	JQ706220
	CPC 16550	*Eucalyptus dives*	Australia: Southern Highlands	B.A. Summerell	JQ706098	JQ706141	JQ706181	JQ706221
Harknessia gibbosa	CPC 12473; CBS 120033[ET]	*Eucalyptus delegatensis*	Australia: Tasmania	C. Mohammed	EF110615	JQ706142	JQ706182	EF110615
	CPC 13646	*Eucalyptus delegatensis*	Australia: Tasmania	B.A. Summerell	JQ706099	JQ706143	JQ706183	JQ706222
	CPC 17626	*Acacia pycnantha*	Australia: Victoria	P.W. Crous	JQ706100	JQ706144	JQ706184	JQ706223
	CPC 17627	*Acacia pycnantha*	Australia: Victoria	P.W. Crous	JQ706101	JQ706145	JQ706185	JQ706224
	CPC 17642	*Eucalyptus* sp.	Australia: Victoria	P.W. Crous	JQ706102	JQ706146	JQ706186	JQ706225
	CPC 17676	*Eucalyptus* sp.	Australia: Victoria	P.W. Crous	JQ706103	JQ706147	JQ706187	JQ706226
Harknessia globispora	CPC 12799	*Eucalyptus globulus*	Portugal	A.L. Phillips	JQ706104	–	JQ706188	JQ706227
	CPC 14924	*Eucalyptus* sp.	Portugal	P.W. Crous	JQ706105	JQ706148	JQ706189	JQ706228
	CPC 3710; CBS 111578[ET]	Leaf litter of *Eucalyptus globulus*	Portugal	S. Denman	AY720722	AY720754	AY720785	AY720819
Harknessia hawaiiensis	CPC 10957; CBS 114811	Leaf litter of *Eucalyptus* sp.	Colombia	M.J. Wingfield	AY720723	AY720755	AY720786	AY720820
	CPC 10960; CBS 115650	Leaf litter of *Eucalyptus* sp.	Colombia	M.J. Wingfield	AY720724	AY720756	AY720787	AY720821
	CPC 11013	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	JQ706106	JQ706149	JQ706190	JQ706229
	CPC 113; CBS 110728	Leaves of *Eucalyptus viminalis*	South Africa: Western Cape Province	P.W. Crous	AY720725	AY720757	AY720788	AY720822
	CPC 15003	*Eucalyptus* sp.	Ecuador	A.C. Alfenas	JQ706107	JQ706150	JQ706191	JQ706230
	CPC 180; CBS 111122	Leaves of *Eucalyptus grandis*	South Africa: Mpumalanga	P.W. Crous	AY720726	AY720758	AY720789	AY720823
Harknessia ipereniae	CPC 12480; CBS 120030[ET]	*Eucalyptus leaf litter*	Australia: Western Australia	A. van Iperen	EF110614	JQ706151	JQ706192	EF110614
Harknessia karwarrae	CPC 10928; CBS 115648	Leaves of *Eucalyptus botryoides*	New Zealand	M. Dick	AY720748	AY720780	AY720811	AY720841

Harknessia kleinzeeina	CPC 108; CBS 110729	*Eucalyptus leaf litter*	South Africa: Western Cape Province	P.W. Crous	AY720739	AY720771	AY720802	–
	CPC 16277[ET]	Leaves of *Eucalyptus* sp.	South Africa: Northern Cape Province	Z.A. Pretorius	JQ706108	JQ706152	JQ706193	JQ706231
Harknessia leucospermi	CPC 1373; CBS 775.97[ET]	Leaf litter of *Leucospermum* sp.	South Africa: Western Cape Province	P.W. Crous	AY720727	AY720759	AY720790	AY720824
	CPC 2849; CBS 114150	Seedling of *Leucospermum* sp.	South Africa: Western Cape Province	J.E. Taylor	AY720728	AY720760	AY720791	AY720825
	CPC 5400; CBS 113526	Dead twigs of *Leucospermum praecox*	South Africa: Western Cape Province	S. Lee	AY720729	AY720761	AY720792	AY720826
	CPC 5403; CBS 112620	Dead twigs of unidentified tree (*Proteaceae*)	South Africa: Western Cape Province	S. Lee	AY720730	AY720762	AY720793	AY720827
	CPC 5404; CBS 112619	Dead twigs of *Protea laurifolia*	South Africa: Western Cape Province	S. Lee	AY720731	AY720763	AY720794	–
Harknessia protearum	CPC 5405; CBS 112618[ET]	Leaf litter of *Leucospermum oleaefolium*	South Africa: Western Cape Province	S. Lee	AY720732	AY720764	AY720795	AY720828
	CPC 5406; CBS 112617	Leaf litter of *Leucospermum* sp.	South Africa: Western Cape Province	S. Lee	AY720733	AY720765	AY720796	AY720829
	CPC 5407; CBS 112616	Dead twig of *Leucadendron* sp.	South Africa: Western Cape Province	S. Lee	AY720734	AY720766	AY720797	AY720830
	CPC 5469; CBS 111830	Dead twigs of *Leucospermum* sp.	South Africa: Western Cape Province	S. Lee	AY720735	AY720767	AY720798	AY720831
	CPC 5470; CBS 111831	Dead twigs of *Leucadendron conocarpodendron*	South Africa: Western Cape Province	S. Lee	AY720736	AY720768	AY720799	AY720832
Harknessia pseudohawaiiensis	CPC 13001	Leaves of *Eucalyptus tereticornis*	Australia: New South Wales	A. Carnegie	JQ706109	JQ706153	JQ706194	JQ706232
	CPC 17300	Leaves of *Eucalyptus* sp.	Australia: Queensland	P.W. Crous	JQ706110	JQ706154	JQ706195	JQ706233
	CPC 17379[ET]; CBS 132124	Leaves of *Eucalyptus dunnii*	Australia: New South Wales	A. Carnegie	JQ706111	JQ706155	JQ706196	JQ706234
Harknessia ravenstreetina	CPC 17095[ET]; CBS 132125	Leaf litter of *Eucalyptus* sp.	Australia: Queensland	P.W. Crous & R.G. Shivas	JQ706112	JQ706156	JQ706197	JQ706235
	CPC 17209; CBS 132126	Twigs of thin-leaved *Acacia* sp.	Australia: Queensland	P.W. Crous & R.G. Shivas	JQ706113	JQ706157	JQ706198	JQ706236
Harknessia renispora	CBS 153.71[EI]	Dead leaf of *Melaleuca pubescens*	Australia: Victoria	H.J. Swart	AY720737	AY720769	AY720800	AY720833
	CPC 17163	*Callistemon pinifolius*	Australia: Queensland	P.W. Crous	JQ706114	JQ706158	JQ706199	JQ706237
Harknessia rhabdosphaera	CPC 12455; CBS 122372	*Eucalyptus nitida*	Australia: Tasmania	M. Glen	JQ706115	JQ706159	–	JQ706238
	CPC 12922; CBS 120082[ET]	Leaves of *Corymbia henryi*	Australia: New South Wales	B.A. Summerell	DQ923532	–	–	DQ923532
	CPC 13593	*Eucalyptus michaeliana*	Australia	B.A. Summerell	JQ706116	JQ706160	JQ706200	JQ706239
	CPC 13594	*Eucalyptus michaeliana*	Australia	B.A. Summerell	JQ706117	–	–	–
	CPC 12847; CBS 122373	*Eucalyptus baxteri*	Australia: South Australia	B.A. Summerell	JQ706118	JQ706161	JQ706201	JQ706240
Harknessia sp.	CPC 11153	Leaf litter of *Eucalyptus* sp.	India	W. Gams	JQ706119	JQ706162	JQ706202	–
Harknessia spermatoidea	CPC 13937[ET]; CBS 132127	Leaf litter of *Eucalyptus* sp.	Cyprus	A. van Iperen	JQ706120	JQ706163	JQ706203	JQ706241
Harknessia syzygii	CPC 184; CBS 111124[ET]	*Syzygium cordatum*	South Africa: Limpopo	M.J. Wingfield	AY720738	AY720770	AY720801	AY720834
Harknessia viterboensis	CPC 10843; CBS 115647[ET]	Leaves of *Eucalyptus* sp.	Italy	W. Gams	AY720740	AY720772	AY720803	JQ706242
Harknessia weresubiae	CPC 12718; CBS 132129	*Eucalyptus* sp.	South Africa: Western Cape Province	P.W. Crous	JQ706121	JQ706164	JQ706204	JQ706243
	CPC 17670[EE]; CBS 132128	*Eucalyptus leaf litter*	Australia: Victoria	P.W. Crous, J. Edwards, I.J. Porter & I.G. Pascoe	JQ706122	JQ706165	JQ706205	JQ706244
	CPC 5106; CBS 113075	Leaf litter of *Eucalyptus* sp.	South Africa: Western Cape Province	P.W. Crous & J. Stone	AY720741	AY720773	AY720804	AY720835
	CPC 5107; CBS 113074	Leaf litter of *Eucalyptus* sp.	South Africa: Western Cape Province	P.W. Crous & J. Stone	AY720742	AY720774	AY720805	AY720836
	CPC 5108; CBS 113073	Leaf litter of *Eucalyptus* sp.	South Africa: Western Cape Province	P.W. Crous & J. Stone	AY720743	AY720775	AY720806	AY720837
	CPC 5109	Leaf litter of *Eucalyptus* sp.	South Africa: Western Cape Province	P.W. Crous & J. Stone	AY720744	AY720776	AY720807	AY720838
Wuestneia molokaiensis	CPC 11127	*Eucalyptus globulus*	Spain	M.J. Wingfield	JQ706123	JQ706166	JQ706206	–
	CPC 12373	*Eucalyptus globulus*	Australia: Victoria	I. Smith	JQ706124	JQ706167	–	JQ706245
	CPC 12995	*Eucalyptus mannifera*	Australia	B.A. Summerell	JQ706125	JQ706168	JQ706207	JQ706246
	CPC 13859	*Eucalyptus* sp.	South Africa	P.W. Crous	JQ706126	JQ706169	JQ706208	JQ706247
	CPC 19269	*Eucalyptus cypellocarpa*	Australia: Northern Territory	P.W. Crous	JQ706127	–	–	JQ706248
	CPC 3797; CBS 114877[ET]	*Eucalyptus robusta*	USA: Hawaii	J.D. Rogers	AY720749	AY579335	AY720812	AY720842

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.

[2] ET: ex-type strain; EE: ex-epitype strain; EI: ex-isotype strain.

[3] LSU: partial 28S nrRNA gene; ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrRNA gene; TUB: partial beta-tubulin gene; CAL: partial calmodulin gene.

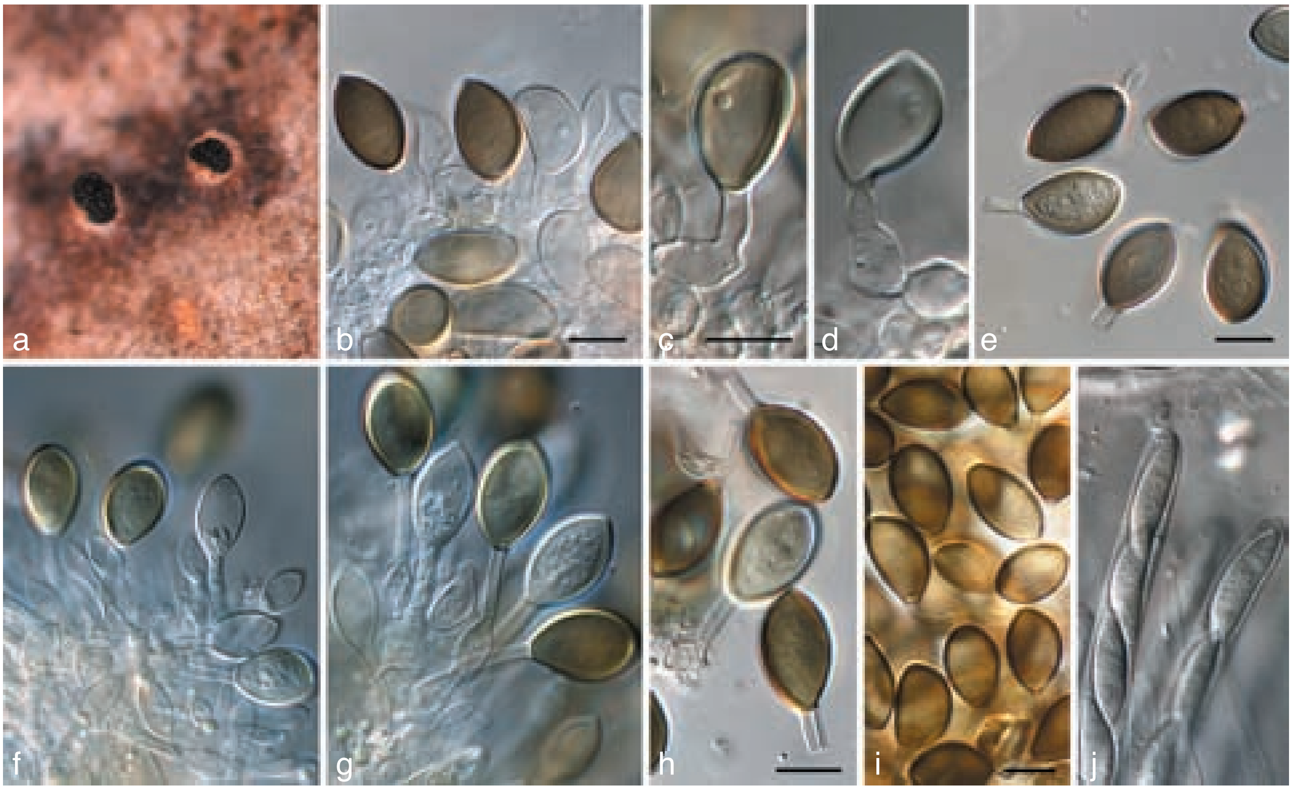


Fig. 3 *Harknessia gibbosa* (CPC 12473). a. Conidiomata sporulating on leaf tissue; b–d, f, g. conidiogenous cells giving rise to conidia; e, h, i. conidia; j. asci of teleomorph. — Scale bars = 10 µm.

RESULTS

DNA phylogeny

The LSU sequences were used to obtain additional sequences from NCBI's GenBank nucleotide database, which were added to the alignment (Fig. 4) and the combined ITS, CAL, and TUB alignment to determine species identification (Fig. 5).

28S nrDNA generic overview

Amplicons of approximately 1 600 bases were obtained for ITS (including the first approx. 900 bp of LSU) of the isolates listed in Table 1. The manually adjusted LSU alignment contained 106 sequences (including the outgroup sequence) and 763 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 164 of these were parsimony-informative, 44 were variable and parsimony-uninformative, and 555 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded tree topologies delimiting similar terminal clades to those of the parsimony analysis (Fig. 4). Only the first 1 000 equally most parsimonious trees were saved (TL = 692 steps; CI = 0.400; RI = 0.842; RC = 0.337).

Bayesian analysis was conducted on the same aligned LSU dataset using a general time-reversible (GTR) substitution model with inverse gamma rates and dirichlet base frequencies. The Markov Chain Monte Carlo (MCMC) analysis of two sets of 4 chains started from a random tree topology and lasted 6 450 000 generations, after which the split frequency reached less than 0.01. Trees were saved each 1 000 generations, resulting in 12 902 saved trees. Burn-in was set at 25 %, leaving 9 678 trees from which the consensus tree (Fig. 5) and posterior probabilities (PP's) were calculated.

A comparison between the tree topologies obtained through the Bayesian, parsimony, and distance analyses yielded mostly the same terminal clades, corresponding to the families as they are delimited in Fig. 4. Some rearrangements are present in the backbone of the tree, for example *Apoharknessia* is intermediate between *Pseudovalsaceae* and *Diaporthaceae* (parsimony, Fig. 4), an unresolved sister clade of *Natarajania indica* basal to *Melanconidaceae* I (distance) or a sister clade to *Pseudovalsaceae* (MrBayes). Similarly, the *Diaporthaceae* and *Valsaceae* are not sister clades (parsimony, Fig. 4), are sister clades with a common node (distance) or are sister clades from a polytomy (MrBayes). The position of *Natarajania indica* also changes with the algorithm used; in parsimony it is a basal sister to *Melanconidaceae* II and *Gnomoniaceae* (Fig. 4), a basal polytomy sister of *Apoharknessia* (distance) or sister to *Gnomoniaceae* (MrBayes). *Schizoparmaceae* is either a direct sister of *Harknessia* (parsimony, Fig. 4), separated from *Harknessia* by *Cryphonectriaceae* (distance) or nestled as a clear lineage in a polytomy of *Harknessia* species (MrBayes). *Cryphonectriaceae* is either a sister clade to *Schizoparmaceae* and *Harknessia* (parsimony, Fig. 4), an intermediate clade between *Schizoparmaceae* and *Harknessia* (distance) or a clade in an unresolved polytomy together with *Natarajania indica*, *Melanconidaceae* II, *Gnomoniaceae*, *Schizoparmaceae*, and *Harknessia* (MrBayes). From the analyses, it is evident that *Cryphonectriaceae*, *Schizoparmaceae* and *Harknessia* are highly similar based on their LSU sequences and that the delimitation of the three clades are sensitive to the algorithm used for the phylogenetic analysis. In all three analyses, *Cryphonectriaceae* is a distinct, well-supported lineage, whereas *Schizoparmaceae* and *Harknessia* form separate clades in the parsimony and distance analyses, albeit without support or poorly supported. In the distance analysis, the bootstrap support values are 62 % for *Harknessia*, 85 % for *Cryphonectriaceae*, and 98 % for *Schizoparmaceae*, 54 % for the association of *Harknessia* and *Cryphonectriaceae*, and 57 % for the branch linking all three clades. The parsimony bootstrap analysis yielded little support for the overall backbone of the tree, although the main families are supported (Fig. 4). However, even more so than in the Bayesian analysis, the *Harknessia* clade collapses to a polytomy with the other families, and *Cryphonectriaceae* and

Schizoparmaceae receive some to good support (58 % and 98 %, respectively).

Species delimitation with combined ITS, *CAL*, *and TUB loci*

Amplicons of approximately 700, 700, and 900 bases were obtained for ITS, CAL, and TUB, respectively, of the isolates listed in Table 1. The manually adjusted combined alignment contained 70 sequences (including the outgroup sequence) and 1 829 characters (614, 505, and 710 characters, respectively) including alignment gaps (available in TreeBASE) which were used in the phylogenetic analysis; 463 of these were parsimony-informative, 393 were variable and parsimony-uninformative, and 973 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded trees with similar topologies to those of the parsimony analysis (Fig. 5). Only the first 1 000 equally most parsimonious trees were saved (TL = 1 813 steps; CI = 0.673; RI = 0.850; RC = 0.572). While many species clades are well-defined, the intraspecific variation for some species such as *H. australiensis*, *H. fusiformis*, *H. renispora*, and *H. rhapdosphaera* appear to be larger than the interspecific variation in the genus (Fig. 5) and these species probably represent species complexes which require the collection of more strains and further study. Other results are discussed under the species notes below, where applicable.

Taxonomy

Harknessiaceae Crous, *fam. nov.* — MycoBank MB564740

Typus. *Harknessia* Cooke, Grevillea 9: 85. 1881.

Mycelium internal, branched, septate, hyaline to pale brown. *Conidiomata* eustromatic to pycnidial, immersed, globose, unilocular to convoluted and multilocular, brown; walls composed of thin-walled, pale brown to brown *textura angularis*. *Ostiolar opening* central, circular, wide, surrounded by brown furfuraceous cells. *Conidiophores* lining the inner cavity, or limited to a basal layer in some species; usually reduced to conidiogenous cells, rarely septate and branched; commonly invested in mucus. *Conidiogenous cells* discrete, ampulliform, lageniform, subcylindrical to cylindrical, hyaline, smooth, giving rise to macroconidia, and in some cases also microconidia in the same conidioma, proliferating one to several times percurrently; secession rhexolytic. *Macroconidia* consisting of a conidium body and a basal appendage, delimited by a septum; conidium body unicellular, of various shapes, thick-walled, smooth, brown, with or without light and dark coloured longitudinal bands, occasionally longitudinally striate, guttulate; basal appendage cellular, cylindrical to subcylindrical, hyaline, flexuous, thin-walled and devoid of contents; apical appendage mostly lacking, when present elongated, attenuated; in some species the conidium body and basal appendage are invested in a thin layer of mucus. *Microconidia* oval to ellipsoid, aseptate, hyaline, smooth. *Ascomata* perithecial, single or aggregated, immersed, disc furfuraceous brown, neck emergent to depressed; wall of 3–5 layers of brown *textura angularis*. *Asci* unitunicate, cylindrical to clavate, hyaline, smooth, 8-spored, with apical apparatus. *Paraphyses* hyaline, septate, interspersed among asci. *Ascospores* aseptate, uni- to biseriate, ellipsoid to fusoid, hyaline, thick-walled, guttulate, smooth.

Notes — The *Cryptosporellaceae*, erected for *Cryptosporella*, is based on *C. hypodermia*, a species having a *Disculina* anamorph (Reid & Booth 1989), thereby making *Cryptosporella* (= *Winterella*) unavailable for *Harknessia* teleomorphs. The genus *Wuestneia*, based on *W. aurea* (= *Wuestneia xanthostroma*), seems an unlikely home for the *Wuestneia*/*Harknessia* complex, as Reid & Booth (1989) found it was associated with a coelomycete anamorph having hyaline conidia. Given the confusion that exists over the genus most suitable for *Harknessia* teleomorphs, the best option is to use a single generic name *Harknessia* (Hawksworth et al. 2011, Wingfield et al. 2012), based on *H. eucalypti*, and introduce *Harknessiaceae* (*Diaporthales*) as a family for these taxa.

Harknessia australiensis Crous & Summerell, *sp. nov*. — MycoBank MB564741; Fig. 6

Etymology. Named after the country where it was collected, Australia.

Foliicolous, isolated from leaves incubated in moist chambers (presumed endophyte). *Conidiomata* pycnidioid, stromatic, amphigenous, scattered, subepidermal, becoming erumpent, globose, up to 300 µm diam; with irregular opening and border of yellowish, furfuraceous cells; wall of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner conidiomatal cavity. *Conidiogenous cells* 5–10 × 4–6 µm, ampulliform to lageniform, hyaline, smooth, invested in mucilage, proliferating once or twice percurrently near apex. *Conidia* (16–)18–20(–22) × (9–)10–11(–12) µm (av. 19 × 11 µm) in vitro, ellipsoid to broadly ventricose, aseptate, golden brown to olivaceous brown, with acutely rounded apex, non-apiculate, thick-walled, smooth, with longitudinal striations along the whole length of the body, granular to multi-guttulate. *Basal appendage* (1.5–)2–3(–4) × 2.5–3 µm in vitro, hyaline, tubular, smooth, thin-walled, devoid of cytoplasm. *Microconidia* not seen.

Culture characteristics — Colonies spreading, fluffy, with abundant aerial mycelium; surface dirty white to cream; cream in reverse; covering the dish in 1 mo.

Specimens examined. Australia, New South Wales, Gibraltar Range National Park, S29°32'22" E152°17'43", 980 m, on leaves of *Eucalyptus dissita*, 19 Mar. 2008, *B.A. Summerell* (CBS H-20911 holotype, cultures ex-type CPC 15029 = CBS 132119); New South Wales, Woodford, S33°43'30" E150°29'25", on leaves of *Eucalyptus sclerophylla* (NSW616452), 26 June 2007, *B.A. Summerell*, CPC 13596–13598 = CBS 132120.

Notes — Morphologically there is little to separate between *H. ravenstreetina* (which appears to occur on a wide host range) and *H. australiensis* (occurs on different *Eucalyptus* spp.). The main distinguishing features are its conidial shape, with conidia of *H. ravenstreetina* being broadly ventricose, and absence of striations, while those of *H. australiensis* are ellipsoid to broadly ventricose, and have prominent striations. These two species were also phylogenetically distinct (Fig. 2).

Harknessia ellipsoidea Crous, R.G. Shivas & Summerell, *sp. nov.* — MycoBank MB564742; Fig. 7

Etymology. Named after its conidial shape, which is broadly ellipsoid.

Foliicolous, isolated from leaves incubated in moist chambers (presumed endophyte). *Conidiomata* pycnidioid, stromatic, amphigenous, scattered, subepidermal, erumpent, globose, up to 400 µm diam; glabrous with wide ruptured opening and border of yellowish, furfuraceous cells; wall of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner conidiomatal cavity. *Conidiogenous cells* 5–10 × 4–6 µm, ampulliform to lageniform, hyaline, smooth, invested in mucilage, proliferating several times percurrently near apex. *Conidia* (9–)11–12(–13) × 7(–8) µm (av. 11.5 × 7 µm) in vitro, broadly ellipsoid to subglobose, aseptate, brown to dark brown, non-apiculate, thick-walled, smooth, granular to multi-guttulate or with large central guttule, non-striate. *Basal appendage* 1–2(–4) × 2 µm in vitro, hyaline, tubular, smooth, thin-walled, devoid of cytoplasm. *Microconidia* not seen.

Culture characteristics — Colonies spreading, fluffy, with moderate to abundant aerial mycelium; surface dirty white to cream to pale luteous; covering the dish in 1 mo.

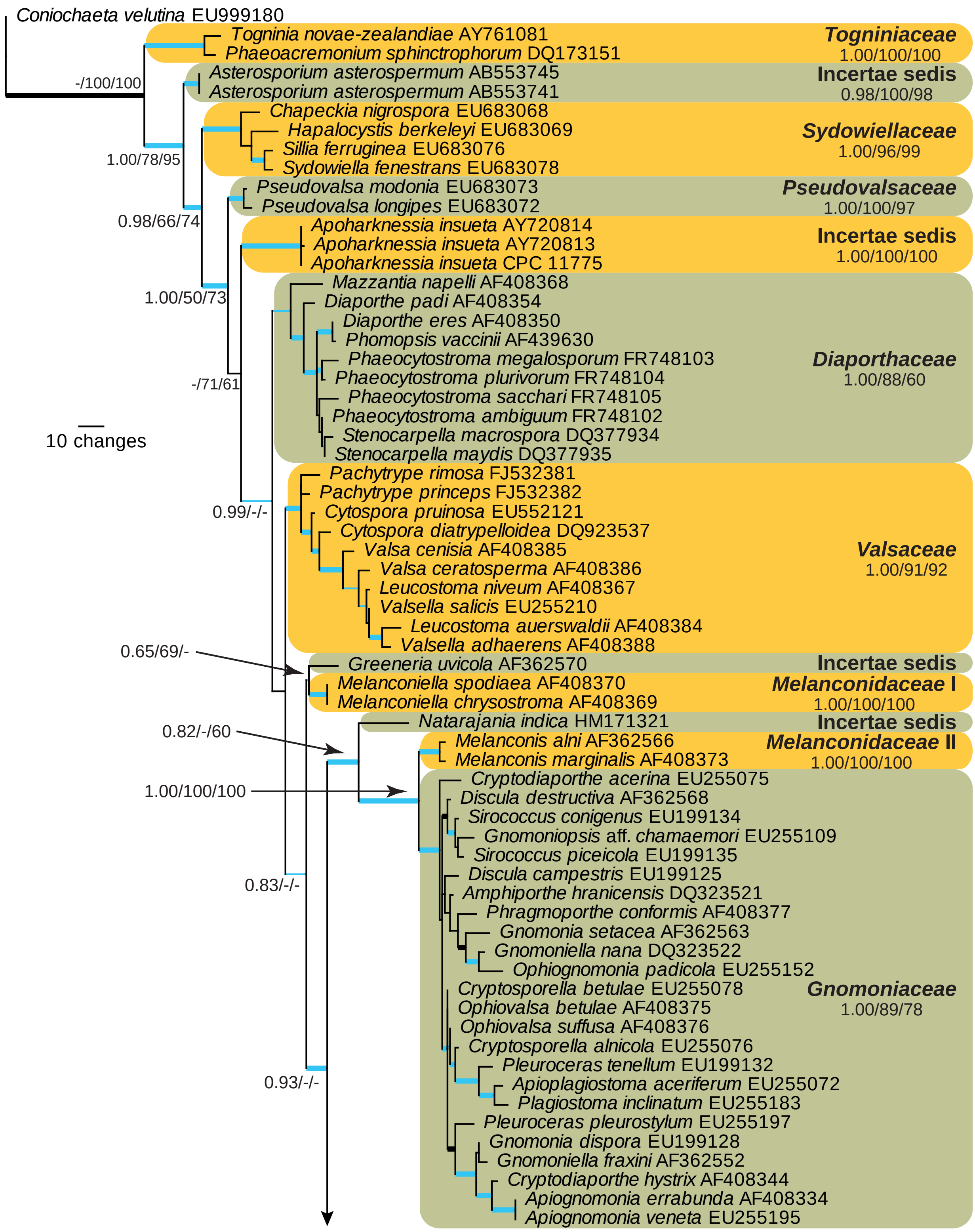
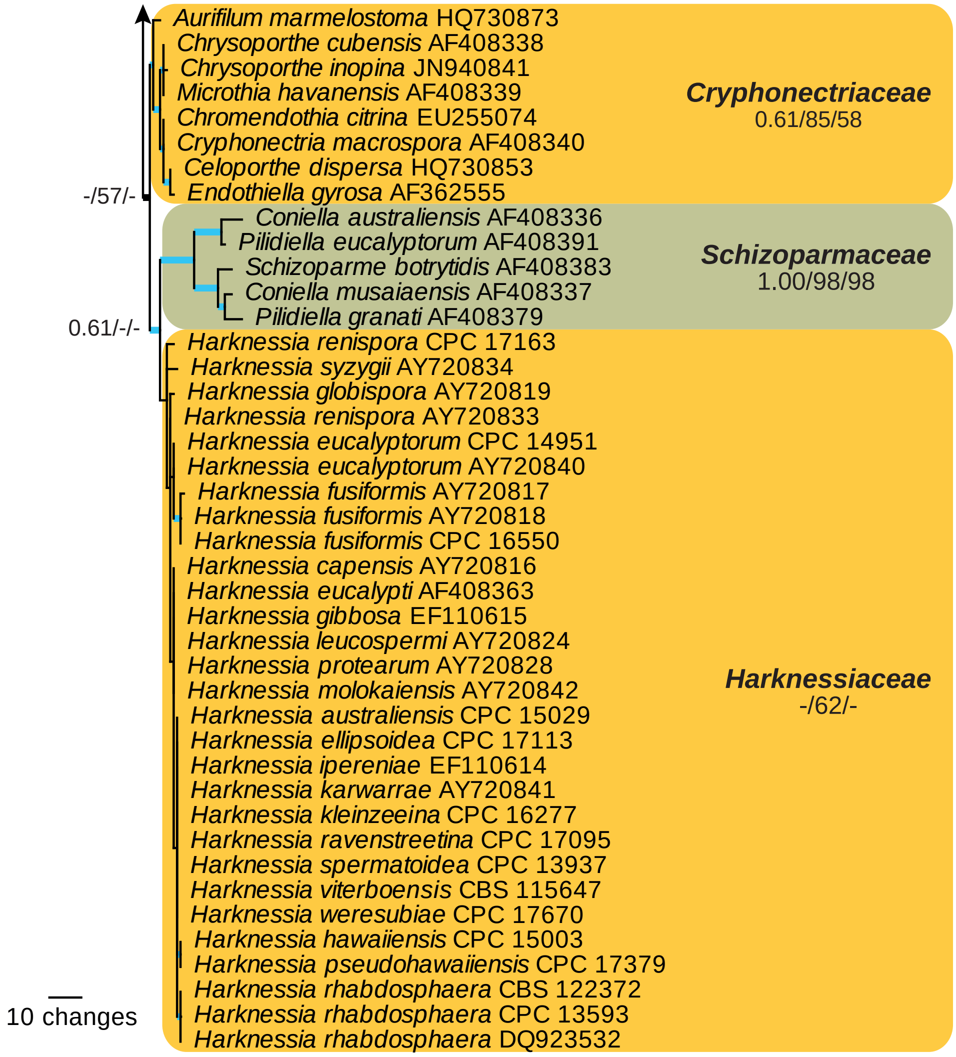


Fig. 4 The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and posterior probability (PP), distance (NJBS), and maximum parsimony (MPBS) bootstrap support values from 1 000 replicates are shown (PP/NJBS/MPBS) for simplicity only for the families and backbone of the phylogenetic tree. Families are indicated to the right of the tree. Branches present in the parsimony strict consensus tree are thickened and those present in both the parsimony consensus and Bayesian tree are drawn in blue. The tree was rooted to a sequence of *Coniochaeta velutina* (GenBank accession EU999180).

Specimens examined. Australia, Queensland, Brisbane, Bardon Trail, on leaves of *Eucalyptus* sp., 12 July 2009, *P.W. Crous & R.G. Shivas* (CBS H-20912 holotype, cultures ex-type CPC 17111 = CBS 132121, CPC 17112, 17113); New South Wales, Kew, S31°42'38" E152°42'20", on leaves of *Eucalyptus propinqua*, 26 Apr. 2006, *B.A. Summerell*, CPC 13077–13079 = CBS 132122.

Notes — This species is phylogenetically distinct from any of the other *Harknessia* species known from sequence data (Fig. 2). Conidia are similar in size to those of *H. pseudohawaiiensis* but differ by being broadly ellipsoidal in shape.

Fig. 4 (cont.)



Harknessia kleinzeeina Crous, *sp. nov*. — MycoBank MB564743; Fig. 8

Etymology. Named after the locality where it was collected in South Africa, Kleinzee.

Foliicolous, associated with irregular leaf spots induced by insect damage, pale brown, but appearing to be secondary infections, probably saprobic. Description on PNA. *Conidiomata* pycnidioid, subepidermal, becoming erumpent, ovoid, black, up to 350 µm diam; dehiscence irregular with wide opening, border with pale yellow furfuraceous cells; wall of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the base of conidiomatal cavity. *Conidiogenous cells* lageniform to subcylindrical, hyaline, smooth, proliferating 1–3 times percurrently near apex, 5–10 × 3–4 µm. *Macroconidia* (20–)22–24(–27) × (11–)12–13 µm (av. 23 × 12 µm) in vitro, composed of a body with basal appendage; body brown, smooth, ellipsoid to oblong-ellipsoid, rarely ventricose, apiculate, aseptate, with longitudinal band of lighter pigment, at times bordered by longitudinal striations covering the length of the conidium body, granular to guttulate, at times with central guttule. *Basal appendage* (30–)45–65(–80) × 2–3 µm in vitro, hyaline, tubular, smooth, thin-walled, flexuous, devoid of cytoplasm, at times walls collapsing, covered in mucilaginous layer when immature. *Microconidia* not seen.

Culture characteristics — Colonies fluffy, spreading with abundant aerial mycelium; surface dirty white to cream or pale luteous; covering the dish in 1 mo; sporulating with black conidiomata, oozing black spore masses.

Specimens examined. South Africa, Northern Cape Province, Kleinzee, on leaves of *Eucalyptus* sp., 27 Feb. 2009, *Z.A. Pretorius* (CBS H-20913 holotype, cultures ex-type CPC 16277 = CBS 132123); Western Cape Province, Stellenbosch Mountain, on *Eucalyptus* leaf litter, 8 Dec. 1988, *P.W. Crous*, PREM 50834, culture CBS 110729 = STE-U 108.

Notes — *Harknessia kleinzeeina* is similar to the type of *H. uromycoides* (basal appendages 57–130 × 2–2.5 µm; Nag Raj 1993), but has shorter basal appendages (30–80 × 2–3 µm). Although originally reported from South Africa as *H. uromycoides* (Crous et al. 1993), Lee et al. (2004) stated that South African strains might well represent a different species within the *H. uromycoides* complex. The collection of a second specimen, which is phylogenetically identical (Fig. 2), supports this hypothesis. Although phylogenetically close to *H. ipereniae*, *H. spermatoidea* and *H. viterboensis*, these species can be distinguished by their CAL and TUB sequences, and less so by their ITS sequences.

Harknessia pseudohawaiiensis Crous & Carnegie, *sp. nov.* — MycoBank MB564744; Fig. 9

Etymology. Named after its morphological similarity to *H. hawaiiensis*.

Foliicolous, isolated from leaves incubated in moist chambers (presumed endophyte). *Conidiomata* pycnidioid, stromatic, amphigenous, scattered, subepidermal, becoming erumpent, globose, up to 400 µm diam; glabrous with wide opening and border of yellowish, furfuraceous cells; wall of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner

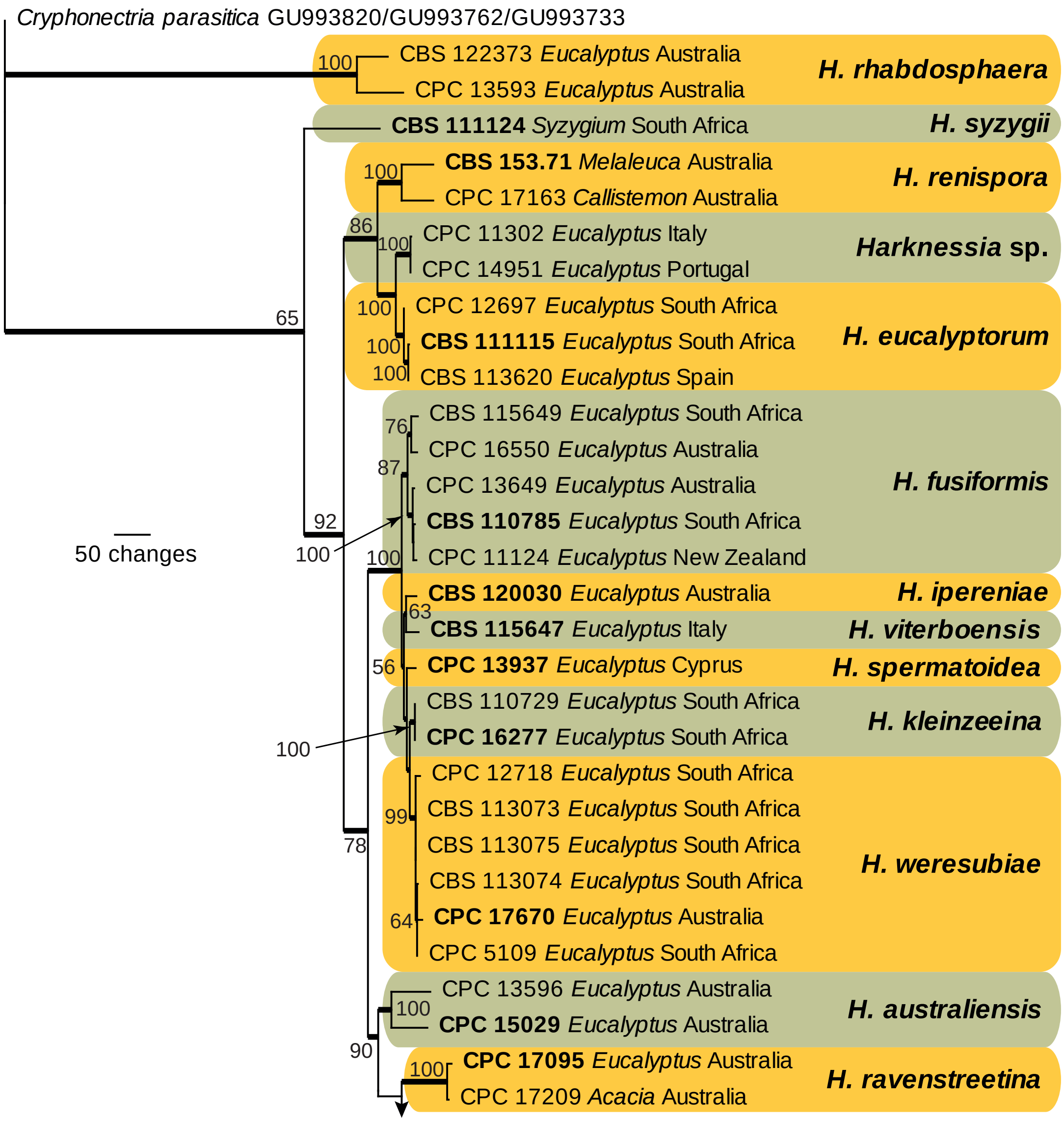


Fig. 5 The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined ITS, CAL, and TUB sequence alignment. The scale bar shows 50 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Ex-type strains are printed in **bold**. Branches present in the strict consensus tree are thickened and the tree was rooted to sequences of *Cryphonectria parasitica* (GenBank ITS: GU993820, CAL: GU993762, TUB: GU993733).

conidiomatal cavity. *Macroconidiogenous cells* 5–9 × 4–6 µm, ampulliform to lageniform, hyaline, smooth, invested in mucilage, proliferating several times percurrently near apex. *Macroconidia* (9–)10–12(–13) × (8–)9(–10) µm (av. 12 × 9 µm) in vitro, subglobose to broadly ellipsoid, aseptate, golden brown to brown, non-apiculate, thick-walled, smooth, granular, with or without longitudinal striations along the length of the body. *Basal appendage* 1–2(–5) × 2 µm in vitro, hyaline, tubular, smooth, thin-walled, devoid of cytoplasm. *Microconidiogenous cells* 4–8 × 4–6 µm, ampulliform to lageniform, hyaline, smooth, with visible apical periclinal thickening. *Microconidia* 4–7 × 2.5–3 µm, hyaline, smooth, fusoid with obtuse apex and tapering to a truncate base.

Culture characteristics — Colonies spreading, fluffy, with moderate to abundant aerial mycelium; surface dirty white to cream to pale luteous; covering the dish in 1 mo.

Specimens examined. Australia, New South Wales, Dundurabbin, Neaves plantation, S30°10'15" E152°30'33", on leaves of *Eucalyptus dunnii*, 22 Sept. 2009, *A.J. Carnegie* (CBS H-20914 holotype, cultures ex-type CPC 17380, 17379 = CBS 132124); Queensland, Cairns Road to Atherton Gillies Highway, on leaves of *Eucalyptus* sp., 16 Aug. 2009, *P.W. Crous*, CPC 17300–17301; New South Wales, Bonalbo, Morpeth Park plantation, S28°46'3" E152°36'47", on leaves of *E. tereticornis*, 30 Mar. 2006, *A.J. Carnegie*, CPC 13001–13003.

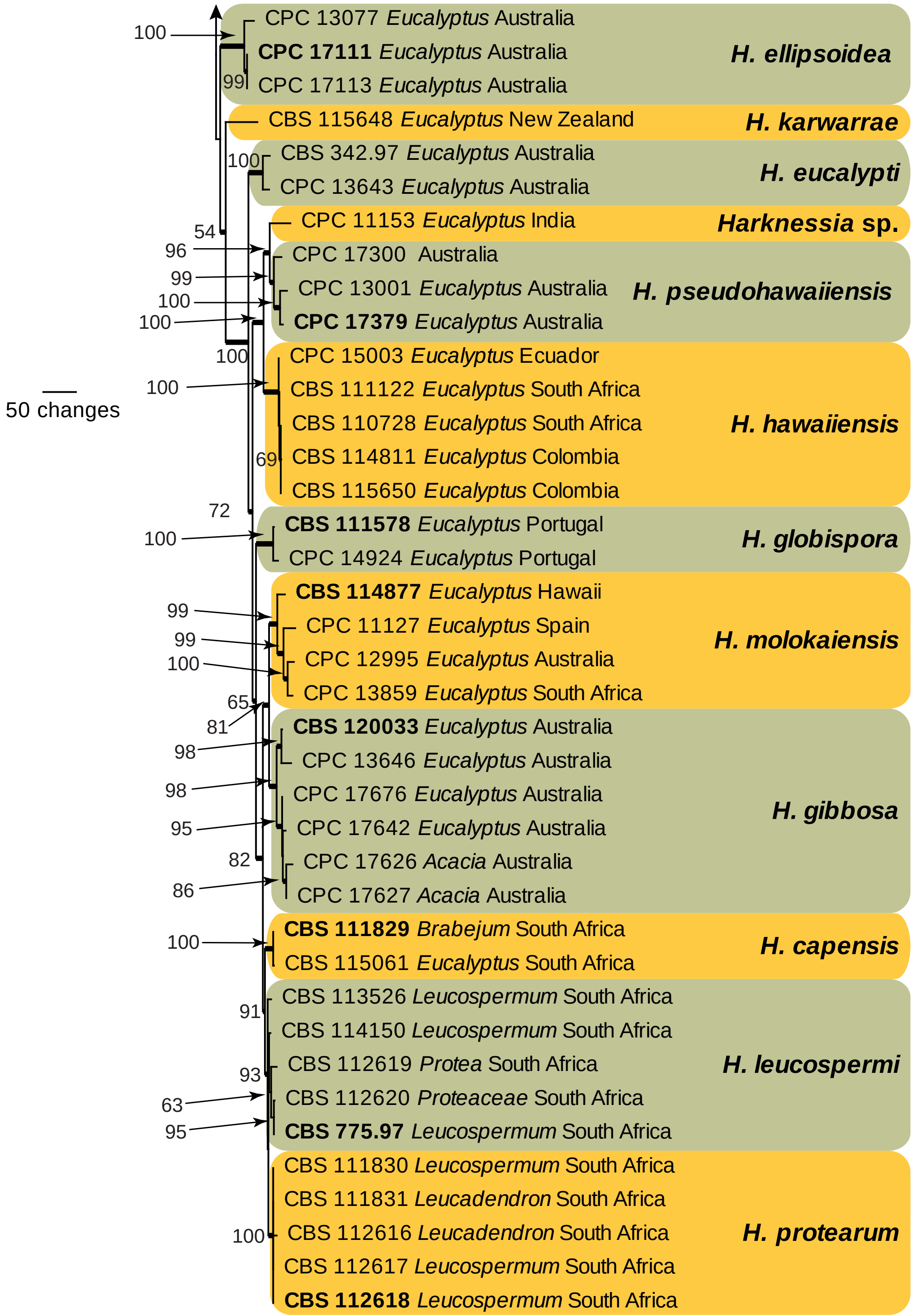
Notes — *Harknessia pseudohawaiiensis* is similar to *H. hawaiiensis* in macroconidial shape, the presence of longitudinal striations, and the abundance of microconidia. It differs in having smaller macroconidia than *H. hawaiiensis* (macroconidia 11–15 × 6.5–8 µm, appendages 2–3 × 2.5 µm), and shorter appendages. These two species are also phylogenetically distinct (Fig. 2). An isolate obtained from *Eucalyptus* in India (on leaf litter of *Eucalyptus* sp., 3 Jan. 2004, *W. Gams*, CPC 11153–11154) appears to represent a closely allied species.

Harknessia ravenstreetina Crous & R.G. Shivas, *sp. nov.* — MycoBank MB564745; Fig. 10

Etymology. Named after the location where it was collected, Raven Street Reserve, Brisbane, Australia.

Caulicolous and *foliicolous*, isolated from leaves and twigs incubated in moist chambers (presumed endophyte). *Conidiomata* pycnidioid, separate to gregarious, subepidermal, becoming erumpent, stromatic, amphigenous, depressed globose, up to 250 µm diam; with irregular opening and border of yellowish, furfuraceous cells; wall of *textura angularis*. *Conidiophores* reduced to conidiogenous cells lining the inner conidiomatal cavity. *Conidiogenous cells* 6–10 × 4–6 µm, ampulliform to sub-

Fig.5 (cont.)



cylindrical, hyaline, smooth, invested in mucilage, percurrently proliferating once or twice near apex. *Conidia* (14–)16–18(–20) × (7–)8(–9) µm (av. 17 × 9 µm) in vitro, broadly ventricose, apex subobtusely rounded, aseptate, non-apiculate, pale yellow-brown, thick-walled, smooth, lacking striations, multi-guttulate. *Basal appendage* (1.5–)2–3(–5) × 2–2.5 µm in vitro, hyaline, tubular, smooth, thin-walled, devoid of cytoplasm. *Microconidia* not seen.

Culture characteristics — Colonies spreading, fluffy, with moderate to abundant aerial mycelium; surface dirty white to cream; cream in reverse; covering the dish in 1 mo.

Specimens examined. Australia, Queensland, Brisbane, Raven Street Reserve, S27°23'22.8" E153°00'16.9" on leaf litter of *Eucalyptus* sp., 12 July 2009, *P.W. Crous & R.G. Shivas* (CBS H-20915 holotype, cultures ex-type CPC 17095 = CBS 132125); Raven Street Reserve, S27°23'22.8" E153°00'16.9" on twigs of thin-leaved *Acacia* sp., 12 July 2009, *P.W. Crous & R.G. Shivas*, cultures CPC 17209 = CBS 132126.

Notes — *Harknessia ravenstreetina* is similar to *H. antarctica* in conidium shape (conidia 20–24 × 10–12 µm, basal appendages 11–28 × 2–3 µm; Nag Raj 1993), although it has smaller conidia, and shorter basal appendages. Unfortunately, a culture of *H. antarctica* was not available for inclusion in the phylogenetic study. *Harknessia ravenstreetina* is phylogenetically distinct from other *Harknessia* species known from sequence data (Fig. 2).

Harknessia spermatoidea R. Galán, G. Moreno & B. Sutton, Trans. Brit. Mycol. Soc. 87: 636. 1986. — Fig. 11

Specimens examined. Cyprus, on leaf litter of *Eucalyptus* sp., salt lake, near airport and Sultan Moskee, 28 Mar. 2007, *A. van Iperen*, CBS H-20924 epitype designated here, culture ex-epitype CPC 13937 = CBS 132127. – Spain, Pontaverda, La Toja, on leaf litter of *Eucalyptus globulus*, 4 Oct. 1985, *N. Manzano*, GM-RG 9320 (holotype), IMI 295508 (isotype).

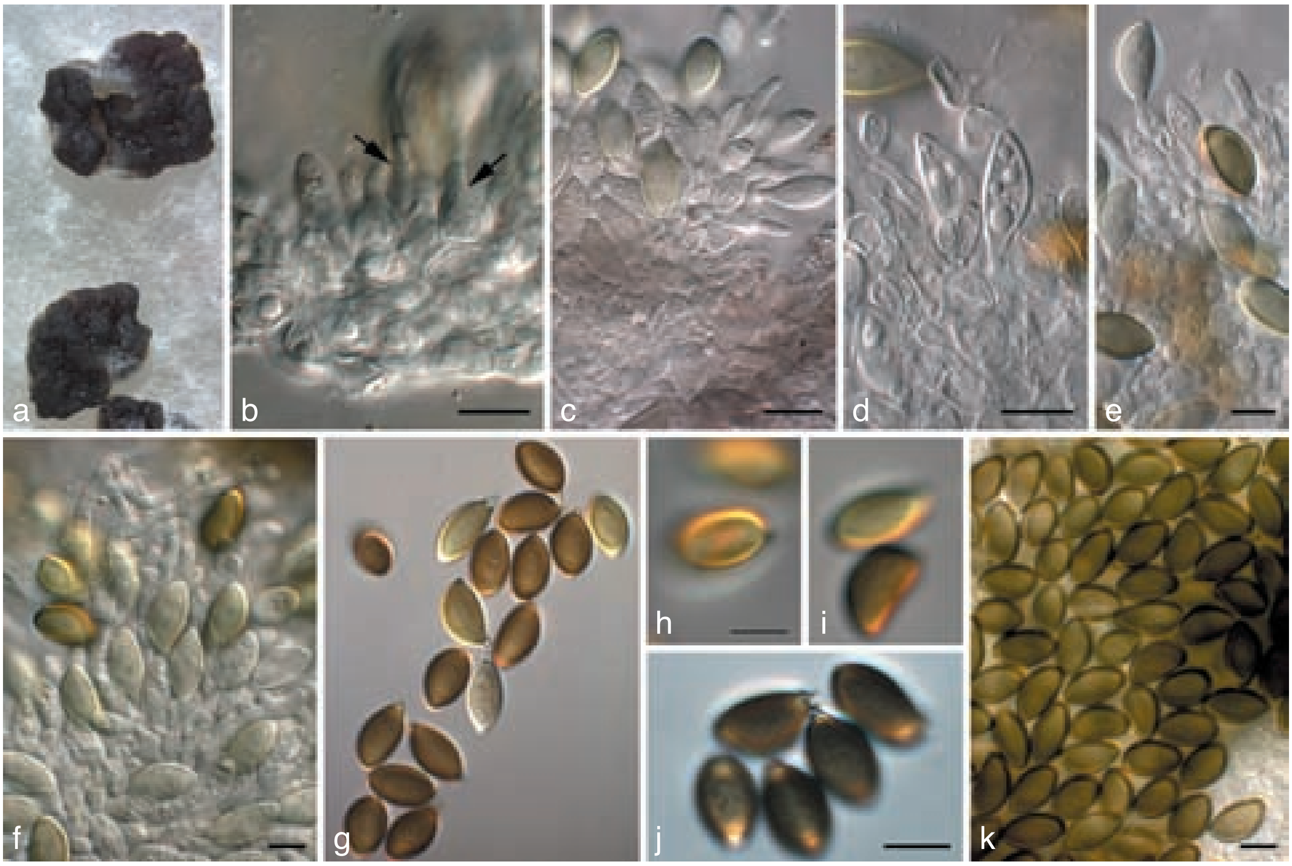


Fig. 6 *Harknessia australiensis* (CPC 15029). a. Sporulating colony on OA; b–f. conidiogenous cells giving rise to conidia (arrows in b denote conidiogenous cells); g–k. conidia with short basal appendages and restricted zones of longitudinal striations. — Scale bars = 10 µm.

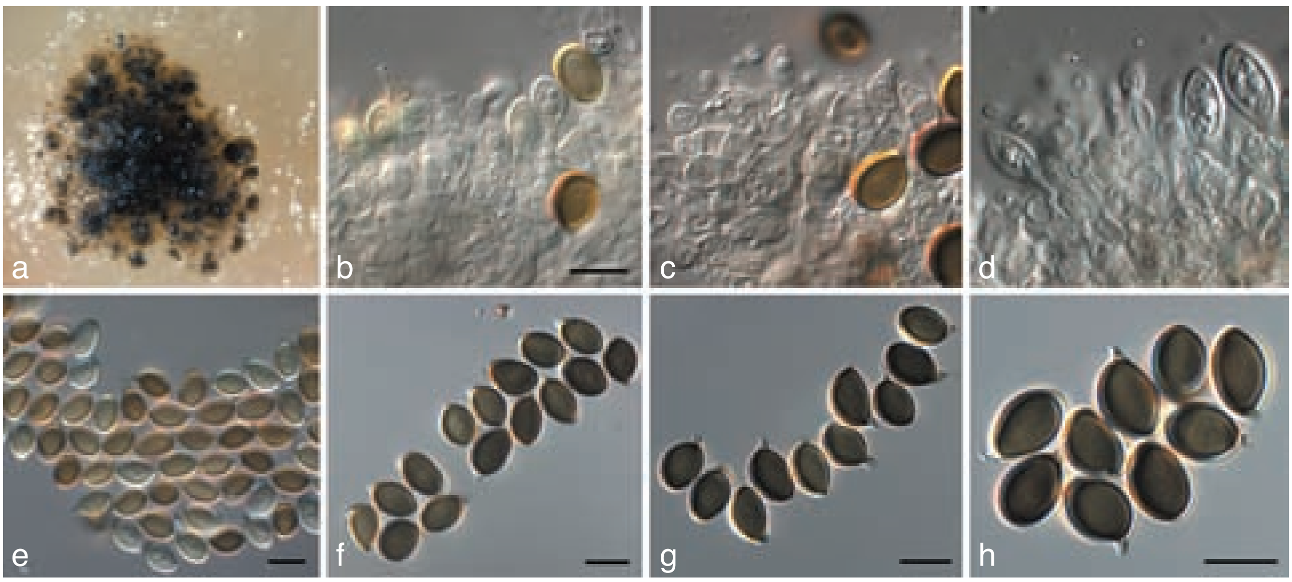


Fig. 7 *Harknessia ellipsoidea* (CPC 17111). a. Sporulating colony on OA; b–d. conidiogenous cells giving rise to conidia; e–h. conidia with short basal appendages. — Scale bars = 10 µm.

Notes — *Harknessia spermatoidea* was originally described from Spain, but the specimen collected on *Eucalyptus* from Cyprus closely matches the morphology observed in the holotype, enabling us to designate an epitype for this taxon. Although phylogenetically closely related to *H. ipereniae*, *H. kleinzeeina*, and *H. viterboensis*, these species can be distinguished by their CAL and TUB sequences, and less easily by their ITS sequences.

Harknessia viterboensis Crous, *sp. nov*. — MycoBank MB564746; Fig. 12

Etymology. Named after the location where it was collected in Italy, Viterbo.

Foliicolous, amphigenous, developing on brown leaf spots after incubation in moist chambers (presumed endophyte). Description on OA, as cultures remained sterile on PNA. *Conidiomata* pycnidioid, erumpent, globose, black, solitary, up to 250 µm diam; dehiscence irregular with wide opening, but generally not exuding excessive amounts of conidia; wall of brown *textura*

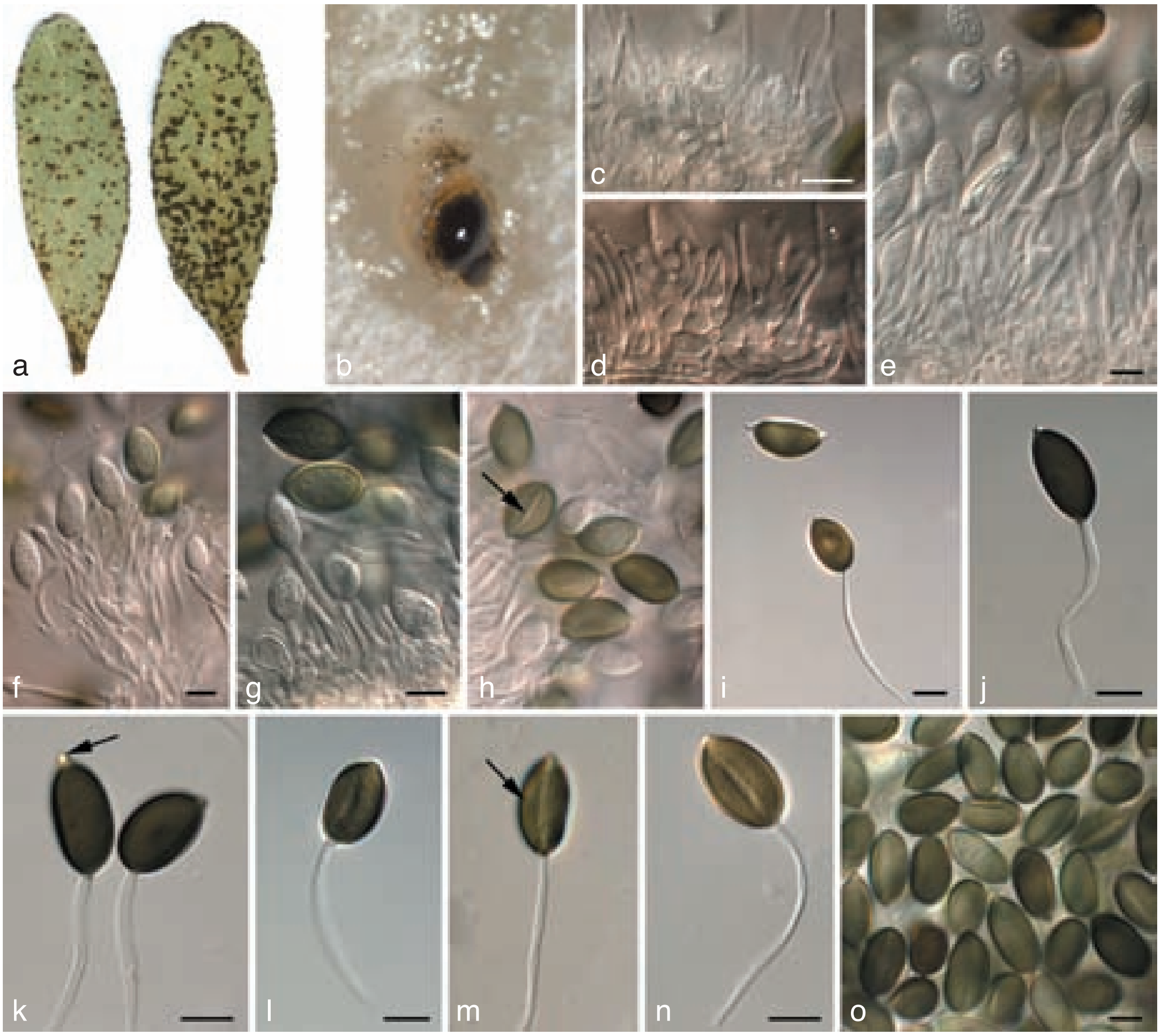


Fig. 8 *Harknessia kleinzeeina* (CPC 16277). a. Insect damage on leaves, creating lesions from which *H. kleinzeeina* was isolated; b. sporulating colony on OA; c–g. conidiogenous cells giving rise to conidia; h–o. conidia with long basal appendages (arrow in k denotes apiculus, and in h and m longitudinal striations). — Scale bars = 10 µm.

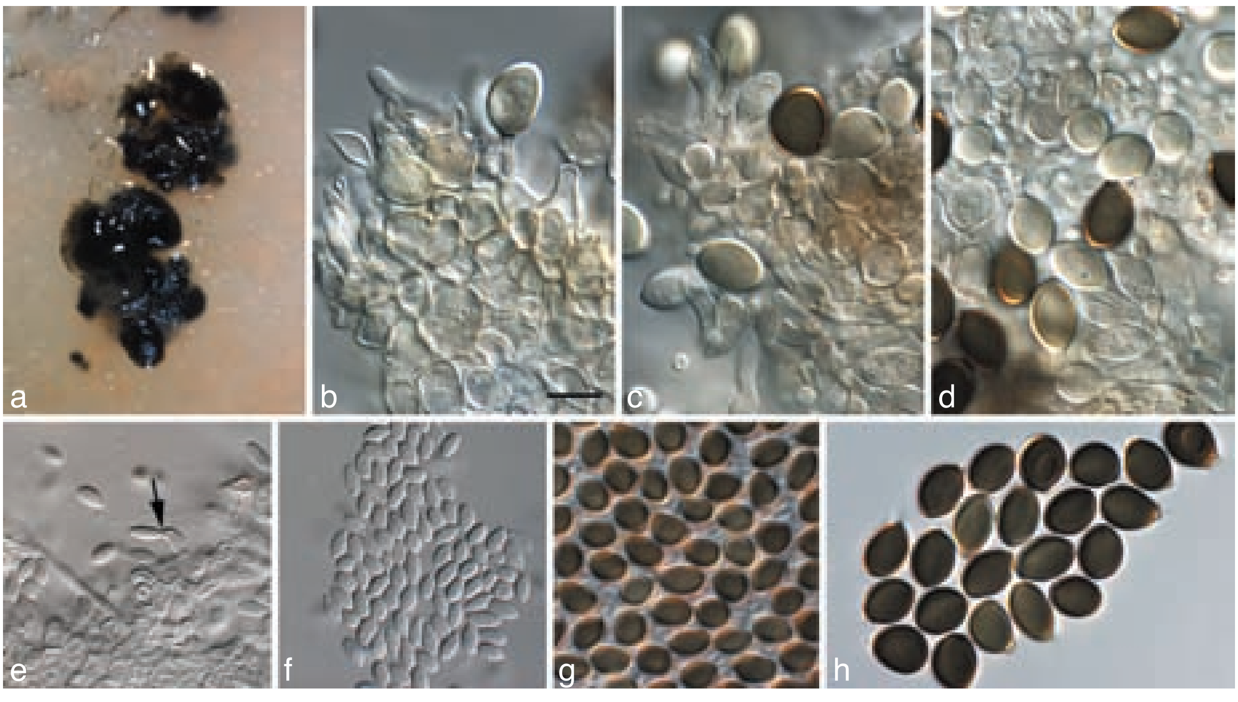


Fig. 9 *Harknessia pseudohawaiiensis* (CPC 17380). a. Sporulating colony on OA; b–d. conidiogenous cells giving rise to conidia; e. microconidiogenous cell giving rise to microconidium (arrow); f. microconidia; g, h. macroconidia. — Scale bars = 10 µm.

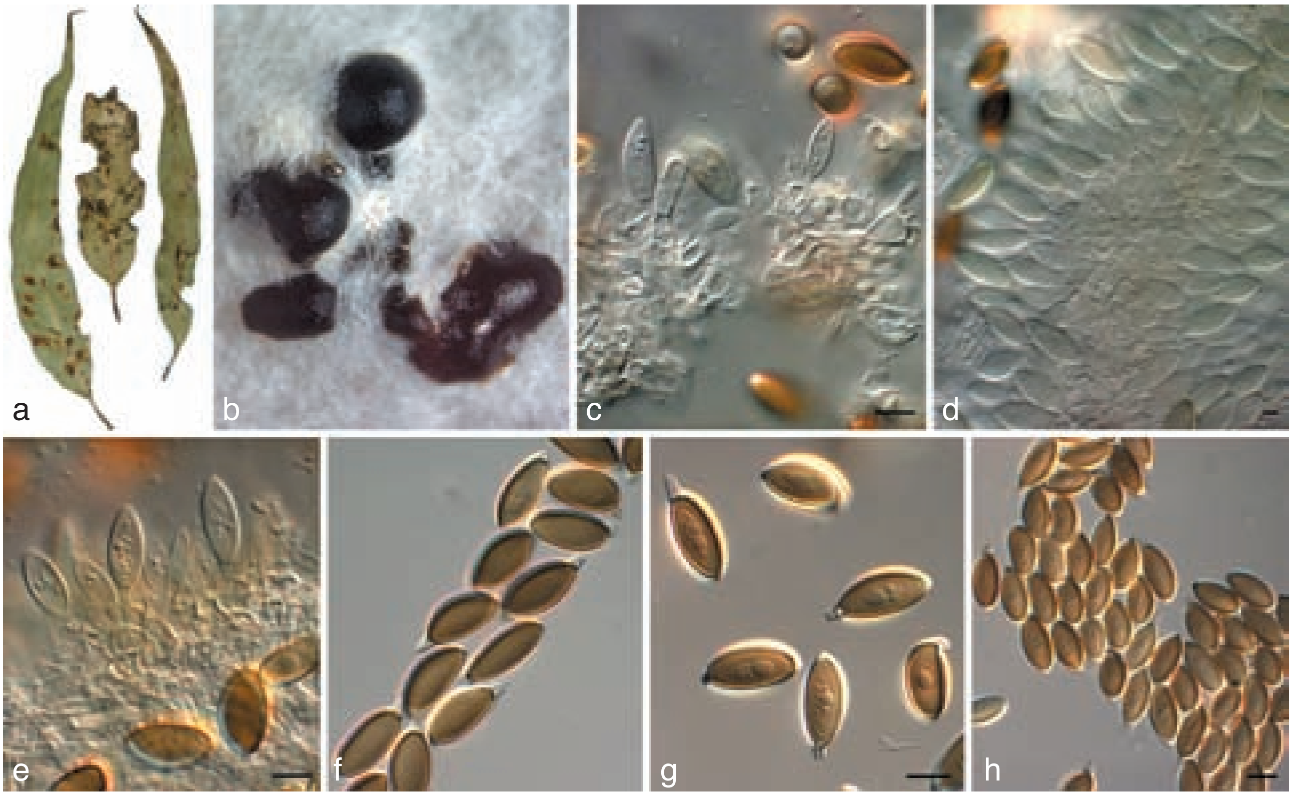


Fig. 10 *Harknessia ravenstreetina* (CPC 17095). a. Leaf spot symptoms on *Eucalyptus*; b. sporulating colony on OA; c–e. conidiogenous cells giving rise to conidia; f–h. conidia. — Scale bars = 10 µm.

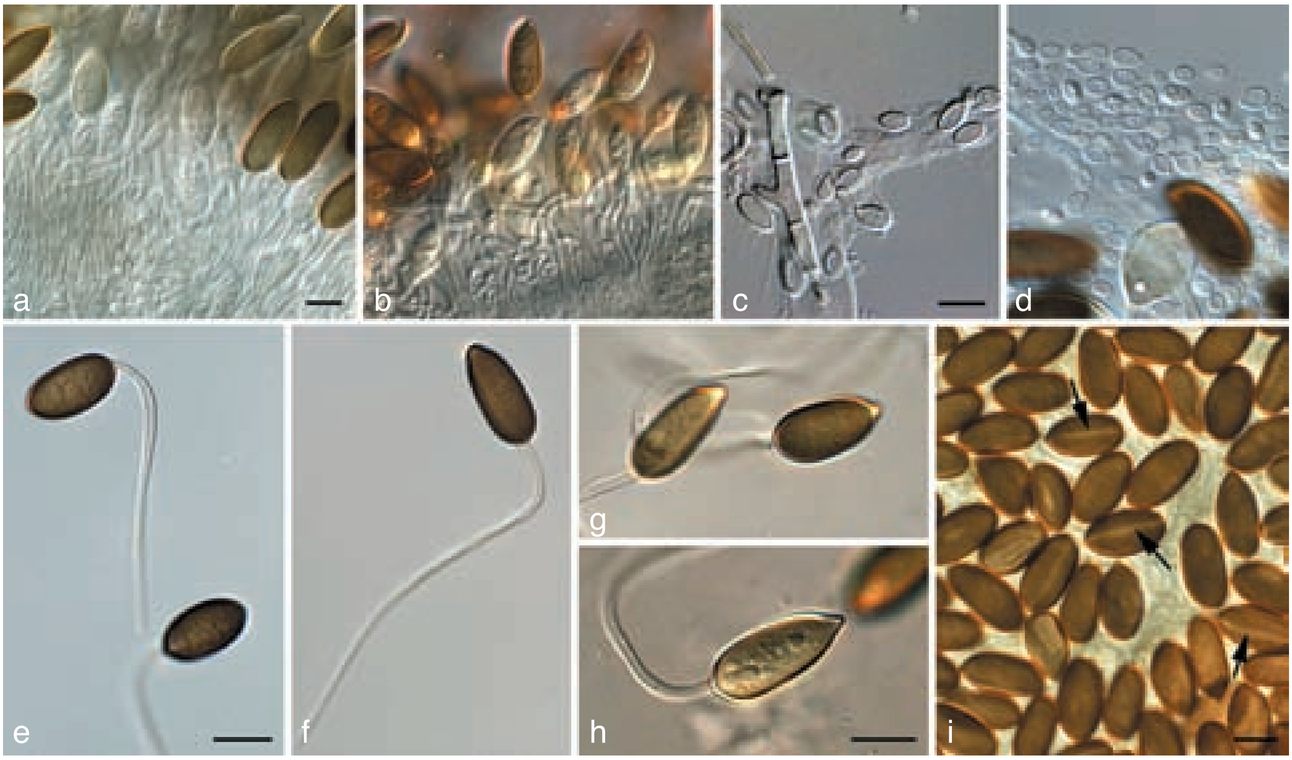


Fig. 11 *Harknessia spermatoidea* (CPC 13937). a, b. Conidiogenous cells giving rise to conidia; c, d. microconidia; e–i. macroconidia with long basal appendages. — Scale bars = 10 µm.

angularis. *Conidiophores* reduced to conidiogenous cells lining the base of conidiomatal cavity, but also forming separately on superficial mycelium. *Conidiogenous cells* ampulliform to lageniform, hyaline, smooth, covered in a mucilaginous layer, holoblastic, rarely proliferating percurrently near apex, 12–20 × 4–6 µm, becoming pale brown with age. *Macroconidia* (17–)20–23(–25) × (9–)10–13(–15) µm (av. 23 × 12 µm) in vitro, composed of a body with basal appendage; body brown to dark brown, smooth, broadly ellipsoid, aseptate, apiculate or apex acutely rounded, aseptate, with longitudinal band of lighter pigment, which can appear like a germ slit in older conidia, at times bordered by longitudinal striations covering the length of the conidium body, multi-guttulate or at times with central guttule. *Basal appendage* (25–)35–60 × (2–)3(–4) µm in vitro, hyaline, tubular, smooth, thin-walled, flexuous, devoid of cytoplasm, at times walls collapsing, covered in mucilaginous layer when im-

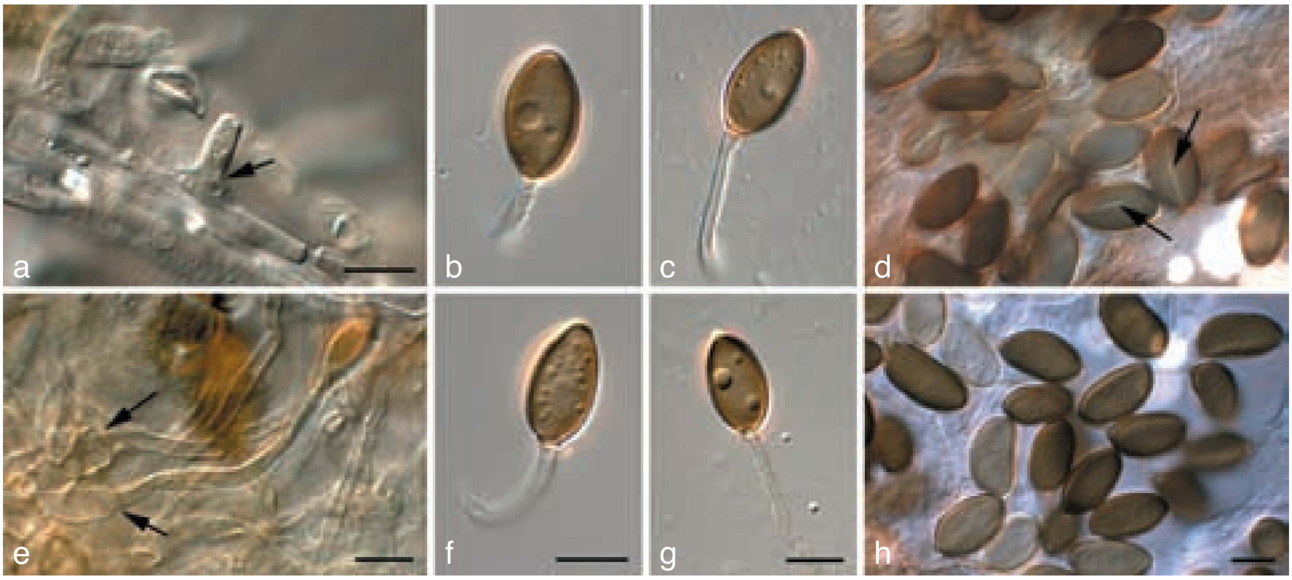


Fig. 12 *Harknessia viterboensis* (CBS 115647). a, e. Conidiogenous cells (arrows); b–d, f–h. conidia with appendages (arrows in d denote apparent germ slit). — Scale bars = 10 µm.

mature; characteristically wide, at times becoming pale brown with age. *Microconidia* not seen.

Culture characteristics — Colonies spreading, somewhat fluffy, with moderate aerial mycelium; surface dirty white to cream; cream in reverse; covering the dish in 1 mo; sporulating poorly, with small, globose olivaceous black conidiomata forming on OA.

Specimen examined. Italy, Viterbo, Vulci, on leaves of *Eucalyptus* sp., Dec. 2003, *W. Gams* (CBS H-9904 holotype, cultures ex-type CPC 10843 = CBS 115647).

Notes — Lee et al. (2004) reported the Italian collection to represent a different species within the *H. uromycoides* complex, but did not formally describe it. It is primarily distinguished from *H. uromycoides* by its shorter and wider appendages, and its prominent longitudinal band of lighter pigment, almost resembling a germ slit. Although phylogenetically related to *H. spermatiodea*, *H. ipereniae* and *H. kleinzeeina*, these species can be distinguished by their CAL and TUB sequences, and less easily by their ITS sequences.

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

Specimens examined. Australia, Saddleworth, on *Eucalyptus* leaf litter, 22 Sept. 1979, *B. Kendrick*, DAOM 173902 (holotype); Victoria, Melbourne, on *Eucalyptus* leaf litter, 21 Oct. 2009, *P.W. Crous*, *J. Edwards*, *I.J. Porter & I.G. Pascoe* (CBS H-20925 epitype designated here, cultures ex-epitype CPC 17670 = CBS 132128). – South Africa, Western Cape Province, Tulbach, on leaf litter of *Eucalyptus* sp., 13 Mar. 2002, *P.W. Crous & J. Stone*, CBS H-9903, cultures CBS 113075 = CPC 5106, CBS 113074 = CPC 5107, CBS 113073 = CPC 5108; Western Cape Province, Malmesbury, on leaf litter of *Eucalyptus* sp., 9 Feb. 2006, *P.W. Crous*, CBS 132129 = CPC 12718–12720.

Notes — *Harknessia weresubiae* occurs on eucalypts in Australia and South Africa (Lee et al. 2004). The species was originally described from Australia, and the fresh Australian collection obtained in the present study enabled us to designate an epitype, and fix the application of the name.

DISCUSSION

The *Diaporthales* is a distinct order within *Sordariomycetes*, a class including perithecial ascomycetous fungi (Zhang & Blackwell 2001, Castlebury et al. 2003). In a recent overview of the order, Rossman et al. (2007) recognised nine families, namely *Sydowiellaceae* (*Sydowiella* and aggregates), *Schizoparmeaceae* (*Schizoparme/Pilidiella* and *Coniella*; van Niekerk et al. 2004), *Gnomoniaceae* (more than 10 sexual genera; Mejía et al. 2011), *Cryphonectriaceae* (*Cryphonectria* generic complex; Gryzenhout et al. 2004, 2006), *Valsaceae* (*Valsa* and aggregates; Castlebury et al. 2002, Adams et al. 2005), *Diaporthaceae* (*Diaporthe/Phomopsis* and aggregates; Mostert et al. 2001, Castlebury et al. 2002, van Rensburg et al. 2006), *Melanconidaceae* (*Melanconis/Melanconium*), *Pseudovalsaceae* (*Pseudovalsa*; Castlebury et al. 2002), and *Togniniaceae* (*Togninia*/*Phaeoacremonium* and *Jobellisia*; Réblová et al. 2004, Mostert et al. 2003, 2006).

Phylogenetic analysis of the LSU sequence data generated in this study resolved a new family in the *Diaporthales*, introduced here as the *Harknessiaceae* (Fig. 4). Morphologically the *Harknessiaceae* is distinct within the order by having *Wuestneia*-like teleomorphs, and pycnidial conidiomata with brown, furfuraceous margins, brown conidia with hyaline, tube-like basal appendages, longitudinal striations, and rhexolytic secession. Furthermore, in addition to previous studies, a multi-gene analysis (ITS, CAL, and TUB), supplemented by morphological criteria, provided additional support to distinguish a further six novel species of *Harknessia* on *Eucalyptus* (Fig. 5), occurring in diverse countries such as Australia, Italy, and South Africa. Although some of these species were clearly associated with leaf spots and are suspected pathogens, many isolates were obtained from asymptomatic leaf tissue, and are presumed to be saprobic.

Although the genus *Harknessia* (type species *H. eucalypti*, teleomorph unknown) was recognised as a separate group in the *Diaporthales* (Castlebury et al. 2002), its family relationships remained unresolved. The main reason for this was that its teleomorph states were placed in *Wuestneia* (Crous et al. 1993, Crous & Rogers 2001). The latter genus is based on *W. xanthostroma*, which has affinities to *Cryphonectriaceae* (Rossman et al. 2007). By establishing the *Harknessiaceae* the correct placement of *Wuestneia* is essentially avoided, as the family is based on the anamorphic genus *Harknessia*, which has *Wuestneia*-like teleomorphs.

Nag Raj (1993) listed several synonyms of *Harknessia*, such as *Caudosporella* (based on *H. antarctica*), *Mastigonetron* (based on *M. fuscum*; having an apical conidial appendage and *Wuestneia*-like teleomorph), and *Cymbothyrium* (based on

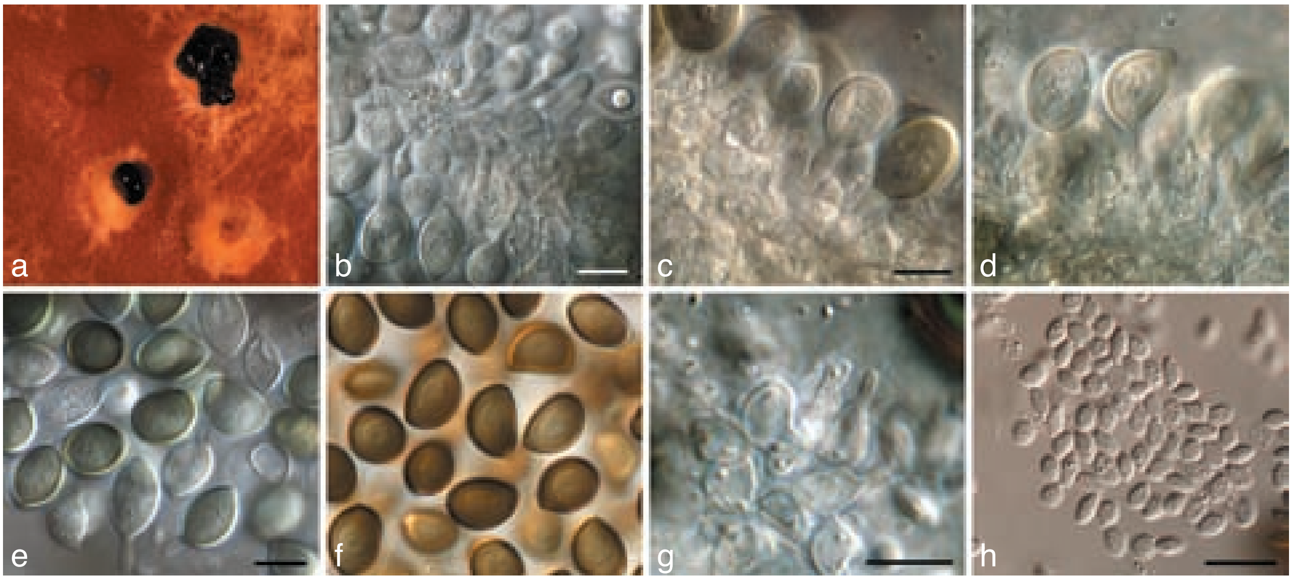


Fig. 13 *Harknessia molokaiensis* (CPC 3797). a. Sporulating colony on MEA; b–d. conidiogenous cells giving rise to macroconidia; e, f. macroconidia; g. microconidiogenous cells giving rise to microconidia; h. microconidia. — Scale bars = 10 µm.

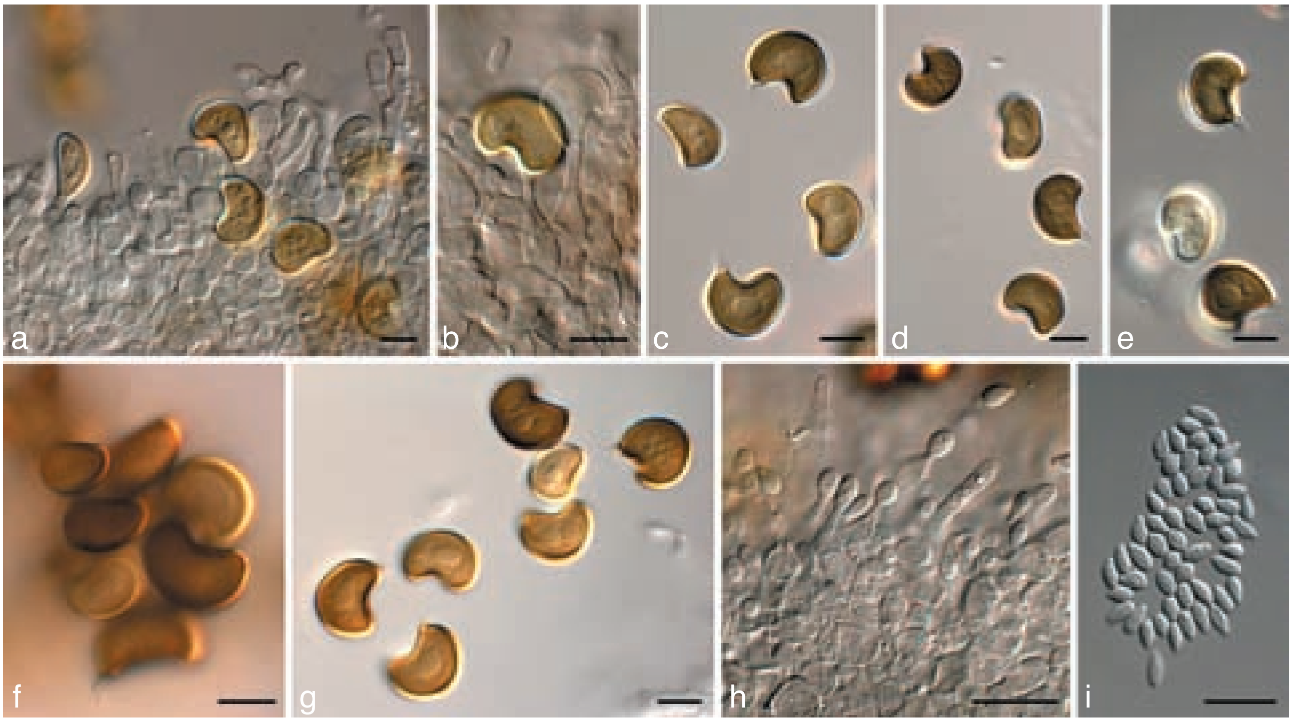


Fig. 14 *Harknessia renispora* (CPC 17163). a, b. Conidiogenous cells giving rise to macroconidia; c–g. macroconidia (not striations in f, and central guttules in g); h. microconidiogenous cells giving rise to microconidia; i. microconidia. — Scale bars = 10 µm.

M. sudans; conidiomata with clypeus). Of these, the synonymy of *Mastigonetron* and *Cymbothyrium* are questionable, but fresh material needs to be collected to facilitate molecular studies to resolve this issue. Other genera that have since been split from *Harknessia* include *Apoharknessia* (with blunt apical appendage; Lee et al. 2004) and *Dwiroopia* (with longitudinal conidial germ slits; Farr & Rossman 2003).

More than 40 species of *Harknessia* have thus far been described, mainly from stems and leaves of angiosperms. Although they are highly variable in morphology and culture characteristics (Fig. 13, 14), they all have brown conidia with basal, cellular appendages. The present study adds an additional six species, and designates epitype specimens for a further two. In spite of extensive collections, the *Harknessiaceae* does not appear to be as species-rich as other families in *Diaporthales*. The addition of fresh collections, and molecular studies conducted on these cultures, will help resolve the uncertainties that remain in *Harknessiaceae*, especially with regards to the host range and distribution of taxa, and the proposed generic synonyms of *Harknessia*.

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