



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

P.W. Crous<sup>1,5,6</sup>, B.A. Summerell<sup>2</sup>, R.G. Shivas<sup>3</sup>, A.J. Carnegie<sup>4</sup>, J.Z. Groenewald<sup>1</sup>

## Key words

biodiversity  
fungal pathogens  
*Harknessiaceae*  
ITS  
LSU  
phylogeny  
systematics

**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. kleinzeina* 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 seces-

sion. Taxa with hyaline conidia and apical appendages were placed in *Mastigospora* (von Höhnelt 1914), while *Apotharknessia* 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 *Caudospora*, *Mastigonetron*, and *Cymbothyrium* (Nag Raj & DiCosmo 1981).

Teleomorphs of *Harknessia* were initially described in *Cryptospora* (Nag Raj & DiCosmo 1981) (*Cryptosporaceae*; 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-

<sup>1</sup> CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl.

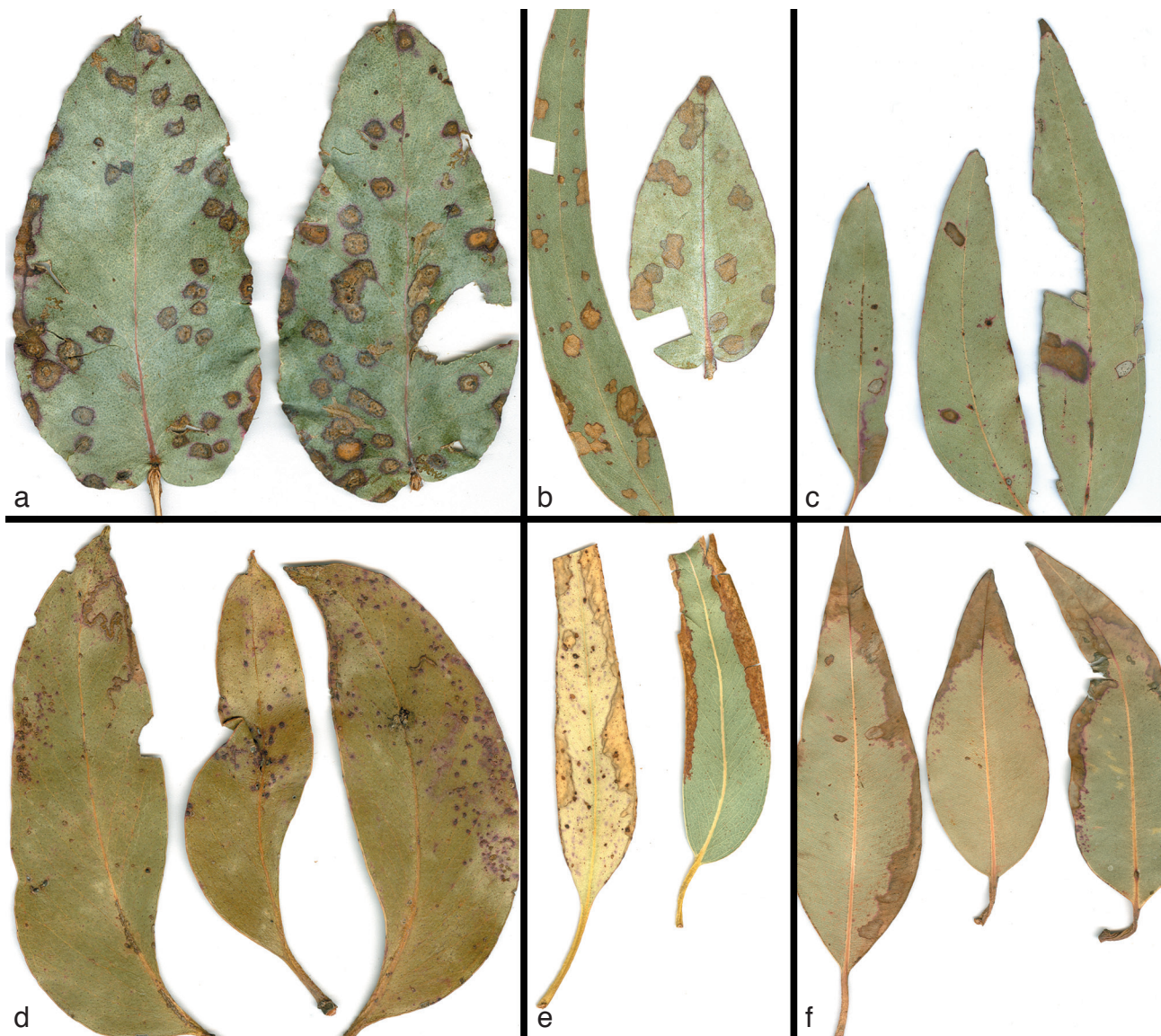
<sup>2</sup> Royal Botanic Gardens and Domain Trust, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.

<sup>3</sup> Plant Biosecurity Science, Department of Employment, Economic Development and Innovation, Ecosciences Precinct, Dutton Park 4102, Queensland, Australia.

<sup>4</sup> Forest Science Centre, NSW Department of Primary Industries, P.O. Box 100, Beecroft, New South Wales 2119, Australia.

<sup>5</sup> Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

<sup>6</sup> Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.



**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 UltraClean™ 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 ( $\times 1\,000$  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. ascogonium with short neck, oozing ascospores; c, d. paraphyses and asci; e–i. asci; j. paraphyse and ascus tip; k, l. asci; m. ascospores; n. conidiomata oozing conidia; o–q. conidia with basal appendages and central guttules. — Scale bars = 10 µm.

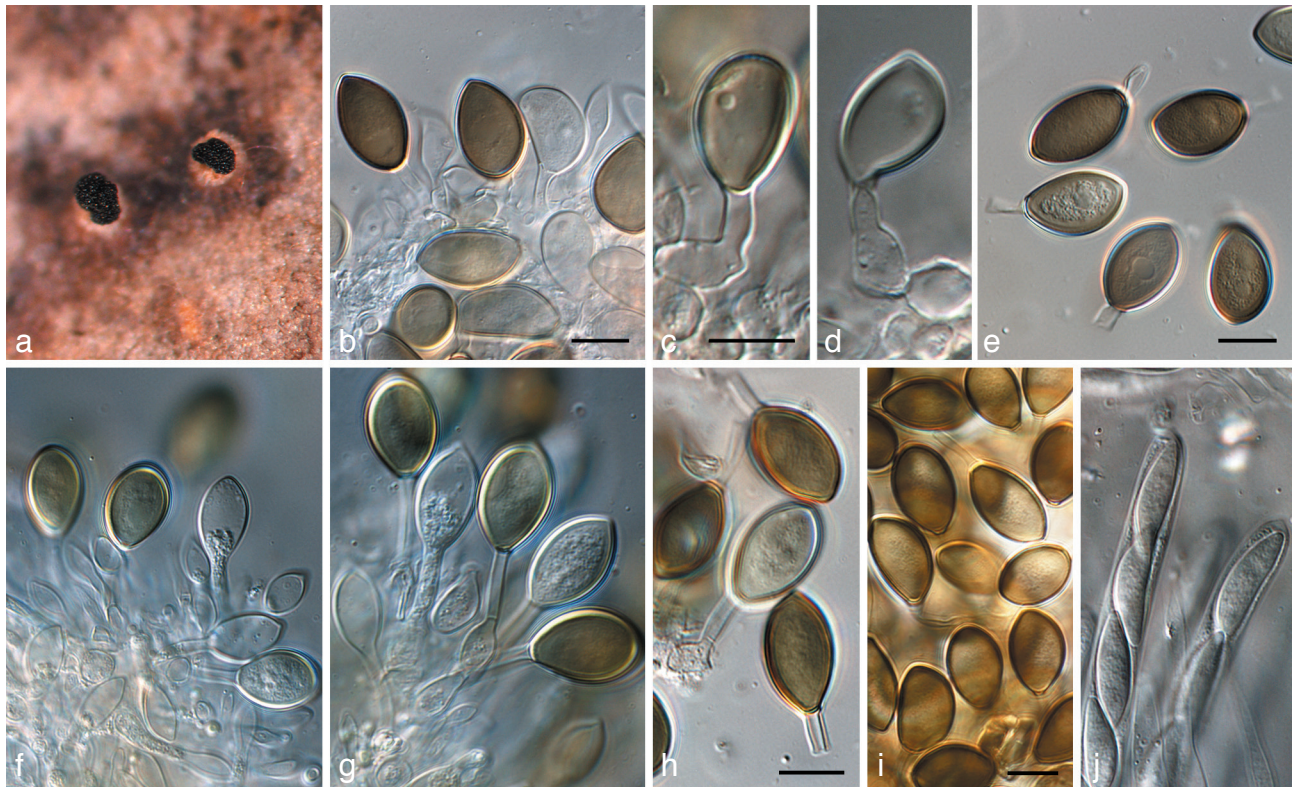
**Table 1** *Harknessia* and *Harknessia*-like isolates included in the morphological and/or phylogenetic analyses.

Species	Culture accession numbers <sup>1,2</sup>	Substrate	Country	Collector	GenBank accession numbers <sup>3</sup>			
					ITS	TUB	CAL	LSU
<i>Apoarknessia insueta</i>	CPC 10947; CBS 114575 CPC 11775 CPC 1451; CBS 111377 <sup>ET</sup>	Leaf spots on <i>Eucalyptus</i> sp. <i>Yucca elephantipes</i> Leaves of <i>Eucalyptus pellita</i>	Colombia Costa Rica Brazil	M.J. Wingfield A. Igram P.W. Crous	– JQ706082 JQ706083	– – –	– – –	AY720813 JQ706209 AY720814
<i>Foliocryphia eucalypti</i>	CPC 12494; CBS 124779 <sup>ET</sup>	<i>Eucalyptus coccoifera</i>	Australia: Tasmania	C. Mohammed	GQ303276	JQ706128	–	GQ303307
<i>Harknessia australiensis</i>	CPC 13596; CBS 132120 CPC 15029 <sup>ET</sup> ; CBS 132119	Leaves of <i>Eucalyptus sclerophylla</i> Leaves of <i>Eucalyptus dissita</i>	Australia: New South Wales Australia: New South Wales	B.A. Summerell B.A. Summerell	JQ706084 JQ706085	JQ706129 JQ706130	JQ706170 JQ706171	JQ706210 JQ706211
<i>Harknessia capensis</i>	CPC 10867; CBS 115061 CPC 5488; CBS 111829 <sup>ET</sup>	<i>Eucalyptus</i> leaves Dead twigs and leaf litter of <i>Brabejum stellatifolium</i>	South Africa: Western Cape Province South Africa: Western Cape Province	P.W. Crous S. Lee	AY720718 AY720719	AY720750 AY720751	AY720781 AY720782	AY720815 AY720816
<i>Harknessia ellipsoidea</i>	CPC 13077; CBS 132122 CPC 17111 <sup>ET</sup> ; CBS 132121 CPC 17113 <sup>ET</sup>	Leaves of <i>Eucalyptus propinque</i> Leaves of <i>Eucalyptus</i> sp. Leaves of <i>Eucalyptus</i> sp.	Australia: New South Wales Australia: Queensland Australia: Queensland	B.A. Summerell P.W. Crous & R.G. Shivas P.W. Crous & R.G. Shivas	JQ706086 JQ706087 JQ706088	JQ706131 JQ706132 JQ706133	JQ706172 JQ706173 JQ706174	JQ706212 JQ706213 JQ706214
<i>Harknessia eucalypti</i>	CBS 342.97 CPC 13643	<i>Eucalyptus regnans</i> <i>Eucalyptus regnans</i>	Australia: Tasmania Australia: Tasmania	Z.-Q. Yuan B.A. Summerell	AY720745 JQ706089	AY720777 JQ706134	AY720808 JQ706175	AF408363 JQ706215
<i>Harknessia eucalyptorum</i>	CBS 113620 CPC 85; CBS 111115 <sup>ET</sup> CPC 11302 CPC 12697 CPC 13074 CPC 14951 CPC 14954 CPC 19659	Leaves of <i>Eucalyptus</i> sp. Leaves of <i>Eucalyptus andrewsii</i> <i>Eucalyptus</i> sp. Leaf litter of <i>Eucalyptus</i> sp. – <i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp. <i>Eucalyptus cypellocarpa</i>	Spain South Africa: Western Cape Province Italy South Africa: Western Cape Province Italy Portugal Portugal Australia: Northern Territory	P.W. Crous & G. Bills P.W. Crous W. Gams P.W. Crous W. Gams P.W. Crous P.W. Crous P.W. Crous P.W. Crous	AY720746 AY720747 JQ706090 JQ706091 JQ706092 JQ706093 JQ706094 JQ706095	AY720778 AY720779 JQ706135 JQ706136 JQ706137 JQ706138	AY720809 AY720810 JQ706176 JQ706177 – JQ706178	AY720839 AY720840 – JQ706216 JQ706217 JQ706218 – –
<i>Harknessia fusiformis</i>	CPC 295; CBS 110785 <sup>ET</sup> CPC 10488; CBS 115649 CPC 1124 CPC 13649 CPC 16550	Leaf litter of <i>Eucalyptus</i> sp. Leaves of <i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp. <i>Eucalyptus globulus</i> <i>Eucalyptus dives</i>	South Africa: Orange Free State South Africa: Orange Free State New Zealand Australia: Tasmania Australia: Southern Highlands	P.W. Crous P.W. Crous J. Stalpers B.A. Summerell B.A. Summerell	AY720721 AY720720 JQ706096 JQ706097 JQ706098	AY720753 AY720752 JQ706139 JQ706140 JQ706141	AY720784 AY720783 JQ706179 – JQ706220	AY720818 AY720817 – – JQ706221
<i>Harknessia gibbosa</i>	CPC 12473; CBS 120033 <sup>ET</sup> CPC 13646 CPC 17626 CPC 17627 CPC 17642 CPC 17676	<i>Eucalyptus delegatensis</i> <i>Eucalyptus delegatensis</i> <i>Acacia pycnantha</i> <i>Acacia pycnantha</i> <i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp.	Australia: Tasmania Australia: Tasmania Australia: Victoria Australia: Victoria Australia: Victoria	C. Mohammed B.A. Summerell P.W. Crous P.W. Crous P.W. Crous	EF110615 JQ706099 JQ706100 JQ706101 JQ706102 JQ706103	JQ706142 JQ706143 JQ706144 JQ706145 JQ706146 JQ706147	JQ706182 JQ706183 JQ706184 JQ706185 JQ706186 JQ706187	EF110615 JQ706222 JQ706223 JQ706224 JQ706225 JQ706226
<i>Harknessia globispora</i>	CPC 12799 CPC 14924 CPC 3710; CBS 111578 <sup>ET</sup>	<i>Eucalyptus globulus</i> <i>Eucalyptus</i> sp. Leaf litter of <i>Eucalyptus globulus</i>	Portugal Portugal Portugal	A.L. Phillips P.W. Crous S. Denman	JQ706104 JQ706105 AY720722	– JQ706148 AY720754	JQ706188 JQ706189 AY720785	JQ706227 JQ706228 AY720819
<i>Harknessia hawaiiensis</i>	CPC 10957; CBS 114811 CPC 10960; CBS 115650 CPC 11013 CPC 113 CPC 130728 CPC 15003 CPC 180; CBS 111122	Leaf litter of <i>Eucalyptus</i> sp. Leaf litter of <i>Eucalyptus</i> sp. <i>Eucalyptus</i> sp. Leaves of <i>Eucalyptus viminalis</i> <i>Eucalyptus</i> sp. Leaves of <i>Eucalyptus grandis</i>	Colombia Colombia Indonesia South Africa: Western Cape Province Ecuador South Africa: Mpumalanga	M.J. Wingfield M.J. Wingfield M.J. Wingfield P.W. Crous A.C. Alfenas P.W. Crous	AY720723 AY720724 JQ706106 AY720725 JQ706107 AY720726	AY720755 AY720756 JQ706149 AY720757 JQ706150 AY720758	AY720786 AY720787 JQ706190 AY720788 JQ706191 AY720789	AY720820 AY720821 JQ706229 AY720822 JQ706230 AY720823
<i>Harknessia iperieniae</i>	CPC 12480; CBS 120030 <sup>ET</sup>	<i>Eucalyptus</i> leaf litter	Australia: Western Australia	A. van Iperen	EF110614	JQ706151	JQ706192	EF110614
<i>Harknessia karwarrae</i>	CPC 10928; CBS 115648	Leaves of <i>Eucalyptus botryoides</i>	New Zealand	M. Dick	AY720748	AY720780	AY720811	AY720841



<i>Harknessia kleinzeina</i>	CPC 108; CBS 110729 CPC 1627 <sup>ET</sup>	<i>Eucalyptus</i> leaf litter Leaves of <i>Eucalyptus</i> sp.	South Africa: Western Cape Province South Africa: Northern Cape Province	P.W. Crous Z.A. Pretorius	AY720739 JQ706108	AY720771 JQ706152	AY720802 JQ706193	– JQ706231
<i>Harknessia leucospermi</i>	CPC 1373; CBS 775.97 <sup>ET</sup> CPC 2849; CBS 114150 CPC 5400; CBS 113526 CPC 5403; CBS 112620 CPC 5404; CBS 112619	Leaf litter of <i>Leucospermum</i> sp. Seedling of <i>Leucospermum</i> sp. Dead twigs of <i>Leucospermum praecox</i> Dead twigs of unidentified tree ( <i>Proteaceae</i> ) Dead twigs of <i>Protea laurifolia</i>	South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province	P.W. Crous J.E. Taylor S. Lee S. Lee S. Lee	AY720727 AY720728 AY720729 AY720730 AY720731	AY720759 AY720760 AY720761 AY720762 AY720763	AY720790 AY720791 AY720792 AY720793 AY720794	AY720824 AY720825 AY720826 AY720827 –
<i>Harknessia protearum</i>	CPC 5405; CBS 112618 <sup>ET</sup> CPC 5406; CBS 112617 CPC 5407; CBS 112616 CPC 5469; CBS 111830 CPC 5470; CBS 111831	Leaf litter of <i>Leucospermum oleaeifolium</i> Leaf litter of <i>Leucospermum</i> sp. Dead twig of <i>Leucadendron</i> sp. Dead twigs of <i>Leucospermum</i> sp. Dead twigs of <i>Leucadendron conocarpodendron</i>	South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province	S. Lee S. Lee S. Lee S. Lee S. Lee	AY720732 AY720733 AY720734 AY720735 AY720736	AY720764 AY720765 AY720766 AY720767 AY720768	AY720795 AY720796 AY720797 AY720798 AY720799	AY720828 AY720829 AY720830 AY720831 AY720832
<i>Harknessia pseudohawaiiensis</i>	CPC 13001 CPC 17300 CPC 17379 <sup>ET</sup> ; CBS 132124	Leaves of <i>Eucalyptus tereticornis</i> Leaves of <i>Eucalyptus</i> sp. Leaves of <i>Eucalyptus dunii</i>	Australia: New South Wales Australia: Queensland Australia: New South Wales	A. Carnegie P.W. Crous A. Carnegie	JQ706109 JQ706110 JQ706111	JQ706153 JQ706154 JQ706155	JQ706194 JQ706195 JQ706196	JQ706232 JQ706233 JQ706234
<i>Harknessia ravenstreetina</i>	CPC 17095 <sup>ET</sup> ; CBS 132125 CPC 17209; CBS 132126	Leaf litter of <i>Eucalyptus</i> sp. Twigs of thin-leaved <i>Acacia</i> sp.	Australia: Queensland Australia: Queensland	P.W. Crous & R.G. Shivas P.W. Crous & R.G. Shivas	JQ706112 JQ706113	JQ706156 JQ706157	JQ706197 JQ706198	JQ706235 JQ706236
<i>Harknessia renispora</i>	CBS 153.71 <sup>EI</sup> CPC 17163	Dead leaf of <i>Melaleuca pubescens</i> <i>Callistemon pinifolius</i>	Australia: Victoria Australia: Queensland	H.J. Swart P.W. Crous	AY720737 JQ706114	AY720769 JQ706158	AY720800 JQ706199	AY720833 JQ706237
<i>Harknessia rhabdosphaera</i>	CPC 12455; CBS 122372 CPC 12922; CBS 120082 <sup>ET</sup> CPC 13593 CPC 13594 CPC 12847; CBS 122373	<i>Eucalyptus nitida</i> Leaves of <i>Corymbia henryi</i> <i>Eucalyptus michaeliana</i> <i>Eucalyptus michaeliana</i> <i>Eucalyptus baxteri</i>	Australia: Tasmania Australia: New South Wales Australia Australia Australia: South Australia	M. Glen B.A. Summerell B.A. Summerell B.A. Summerell	JQ706115 DQ923532 JQ706116 JQ706117 JQ706118	JQ706159 – JQ706160 – JQ706201	– – JQ706200 – JQ706240	JQ706238 DQ923532 JQ706239 – JQ706240
<i>Harknessia</i> sp.	CPC 11153	Leaf litter of <i>Eucalyptus</i> sp.	India	W. Gams	JQ706119	JQ706162	JQ706202	–
<i>Harknessia spermatoidea</i>	CPC 13937 <sup>ET</sup> ; CBS 132127	Leaf litter of <i>Eucalyptus</i> sp.	Cyprus	A. van Iperen	JQ706120	JQ706163	JQ706203	JQ706241
<i>Harknessia syzygii</i>	CPC 184; CBS 111124 <sup>ET</sup>	<i>Syzygium cordatum</i>	South Africa: Limpopo	M.J. Wingfield	AY720738	AY720770	AY720801	AY720894
<i>Harknessia viterboensis</i>	CPC 10843; CBS 115647 <sup>ET</sup>	Leaves of <i>Eucalyptus</i> sp.	Italy	W. Gams	AY720740	AY720772	AY720803	JQ706242
<i>Harknessia weresubiae</i>	CPC 12718; CBS 132129 CPC 17670 <sup>EE</sup> ; CBS 132128	<i>Eucalyptus</i> sp. <i>Eucalyptus</i> leaf litter	South Africa: Western Cape Province Australia: Victoria	P.W. Crous P.W. Crous, J. Edwards, I.J. Porter & I.G. Pascoe	JQ706121	JQ706164	JQ706204	JQ706243
	CPC 5106; CBS 113075 CPC 5107; CBS 113074 CPC 5108; CBS 113073 CPC 5109	Leaf litter of <i>Eucalyptus</i> sp. Leaf litter of <i>Eucalyptus</i> sp. Leaf litter of <i>Eucalyptus</i> sp. Leaf litter of <i>Eucalyptus</i> sp.	South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province South Africa: Western Cape Province	P.W. Crous & J. Stone P.W. Crous & J. Stone P.W. Crous & J. Stone P.W. Crous & J. Stone	JQ706122 AY720741 AY720742 AY720743	JQ706165 AY720773 AY720774 AY720775	JQ706205 AY720804 AY720805 AY720806	JQ706244 AY720835 AY720836 AY720837 AY720838
<i>Wuestneia molokaiensis</i>	CPC 11127 CPC 12373 CPC 12995 CPC 13859 CPC 19269 CPC 3797; CBS 114877 <sup>ET</sup>	<i>Eucalyptus globulus</i> <i>Eucalyptus globulus</i> <i>Eucalyptus mamifera</i> <i>Eucalyptus</i> sp. <i>Eucalyptus cypellocarpa</i> <i>Eucalyptus robusta</i>	Spain Australia: Victoria Australia South Africa Australia: Northern Territory USA: Hawaii	M.J. Wingfield I. Smith B.A. Summerell P.W. Crous P.W. Crous J.D. Rogers	JQ706123 JQ706124 JQ706125 JQ706126 JQ706127 AY720749	JQ706166 JQ706167 JQ706168 JQ706169 – AY579335	JQ706206 – JQ706207 JQ706208 – AY720812	– JQ706245 JQ706246 JQ706247 JQ706248 AY720842

<sup>1</sup> CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht; The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.<sup>2</sup> ET: ex-type strain; EE: ex-epitype strain; EI: ex-isotype strain.<sup>3</sup> LSU: partial 28S rRNA gene; ITS: internal transcribed spacer regions 1 & 2 including 5.8S rRNA 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 *Apotharknessia* 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 *Apotharknessia* (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. rhapsodisphaera* 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, unicellular to convoluted and multilocular, brown; walls composed of thin-walled, pale brown to brown *textura angularis*. *Ostiole* 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 biseriolate, ellipsoid to fusoid, hyaline, thick-walled, guttulate, smooth.

**Notes** — The *Cryptosporrellaceae*, erected for *Cryptosporrella*, is based on *C. hypodermia*, a species having a *Disculina* anamorph (Reid & Booth 1989), thereby making *Cryptosporrella* (= *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* pycnidoid, 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.

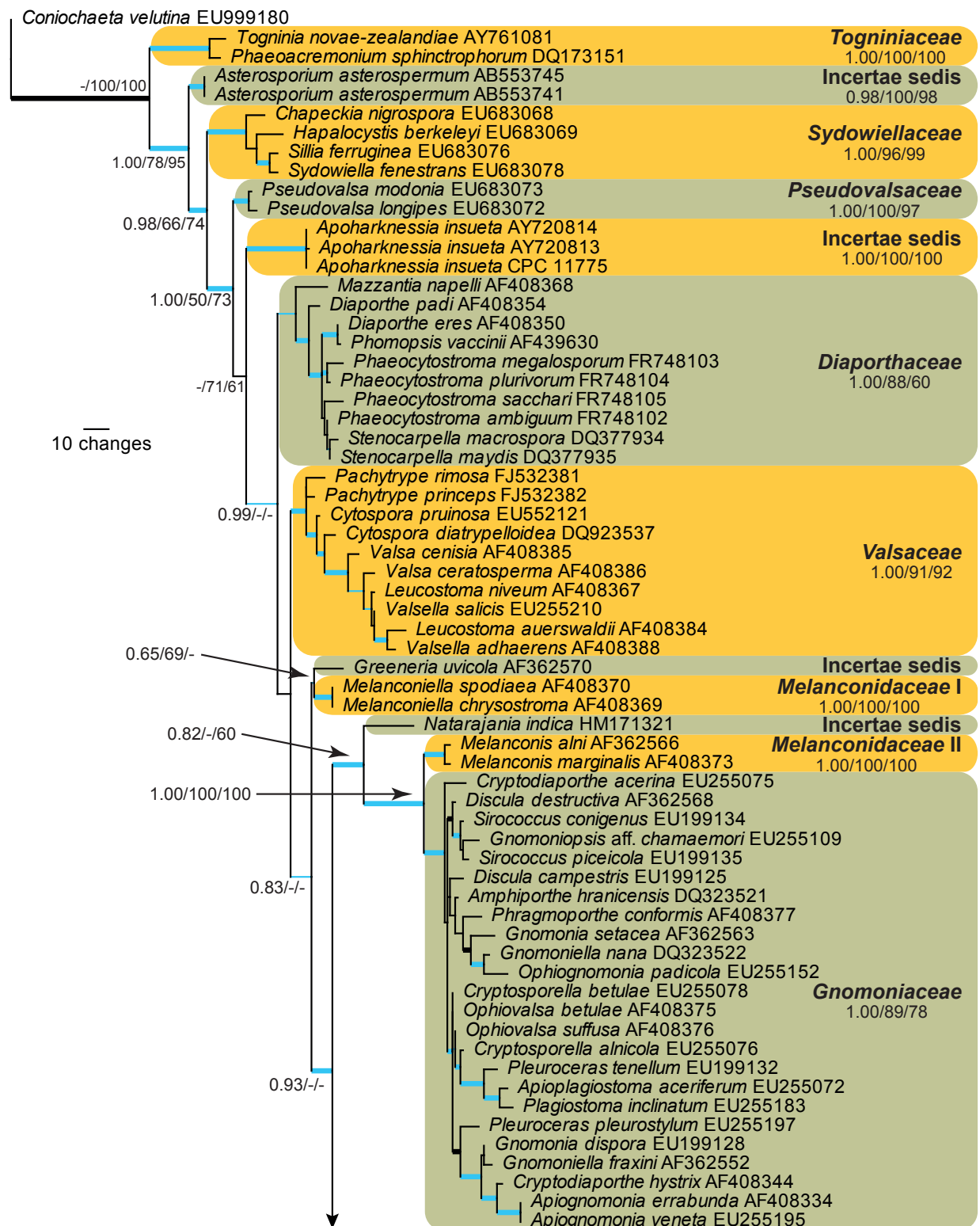
**Notes** — Morphologically there is little to separate between *H. ravenstreetina* (which appears to occur on a wide host range) and *H. australiensis* (occurs on different *Eucalyptus* spp.). The main distinguishing features are its conidial shape, with conidia of *H. ravenstreetina* being broadly 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* pycnidoid, 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.



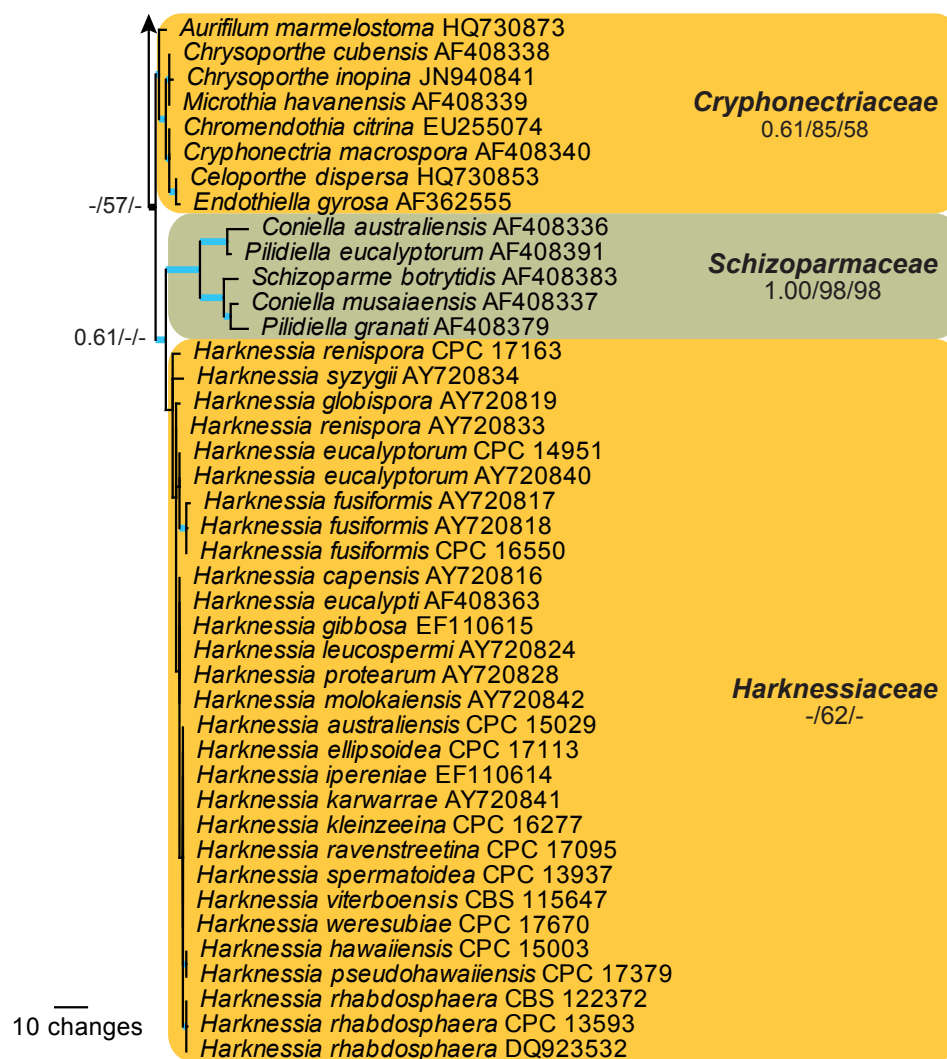
**Fig. 4** The first of 1 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and posterior probability (PP), distance (NJBS), and maximum parsimony (MPBS) bootstrap support values from 1 000 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. pseudo-hawaiiensis* 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* pycnidoid, 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.

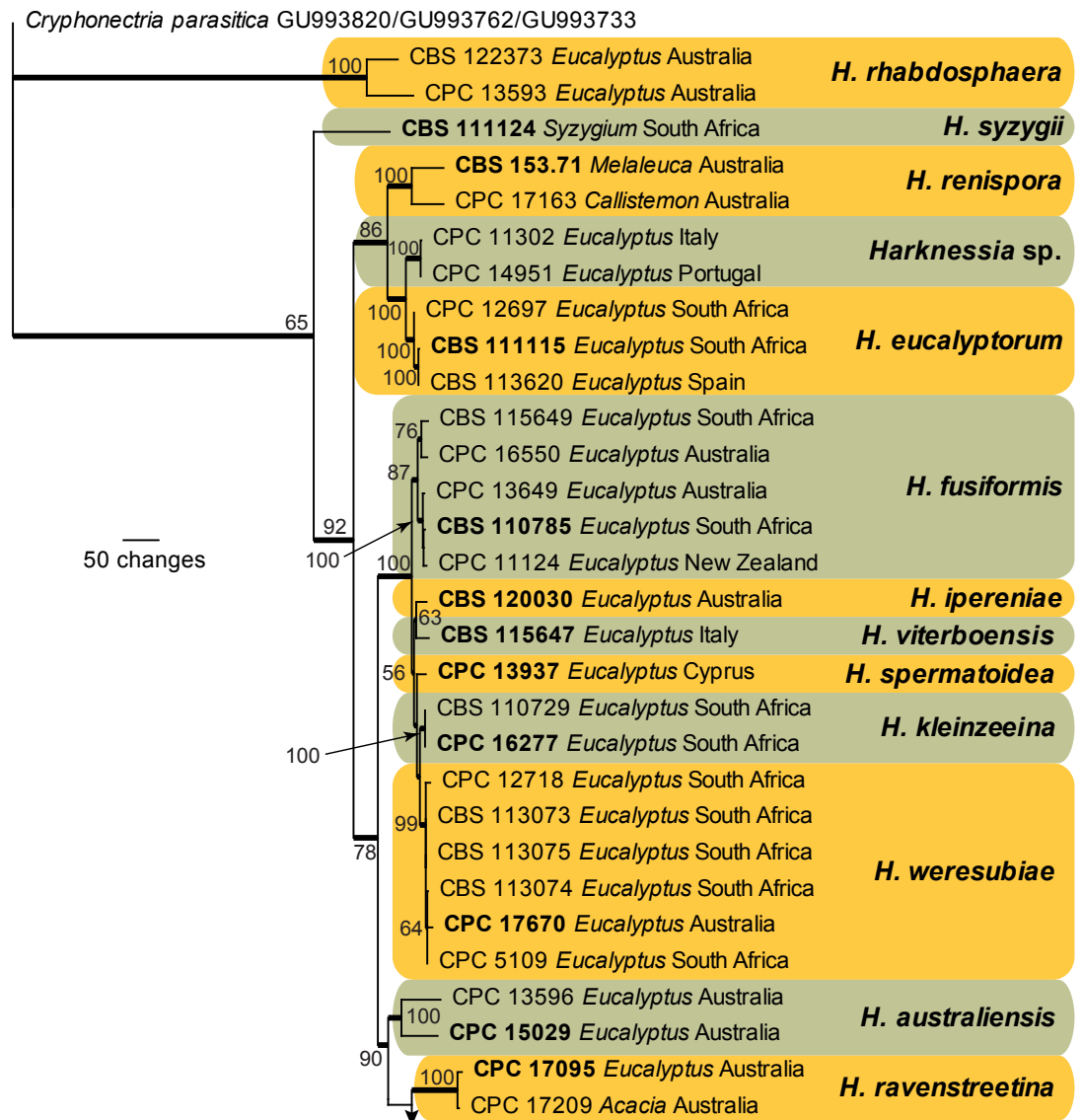
**Specimens examined.** SOUTH AFRICA, Northern Cape Province, Kleinzee, on leaves of *Eucalyptus* sp., 27 Feb. 2009, Z.A. Pretorius (CBS H-20913 holotype, cultures ex-type CPC 16277 = CBS 132123); Western Cape Province, Stellenbosch Mountain, on *Eucalyptus* leaf litter, 8 Dec. 1988, P.W. Crous, PREM 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 1953), 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* pycnidoid, 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.

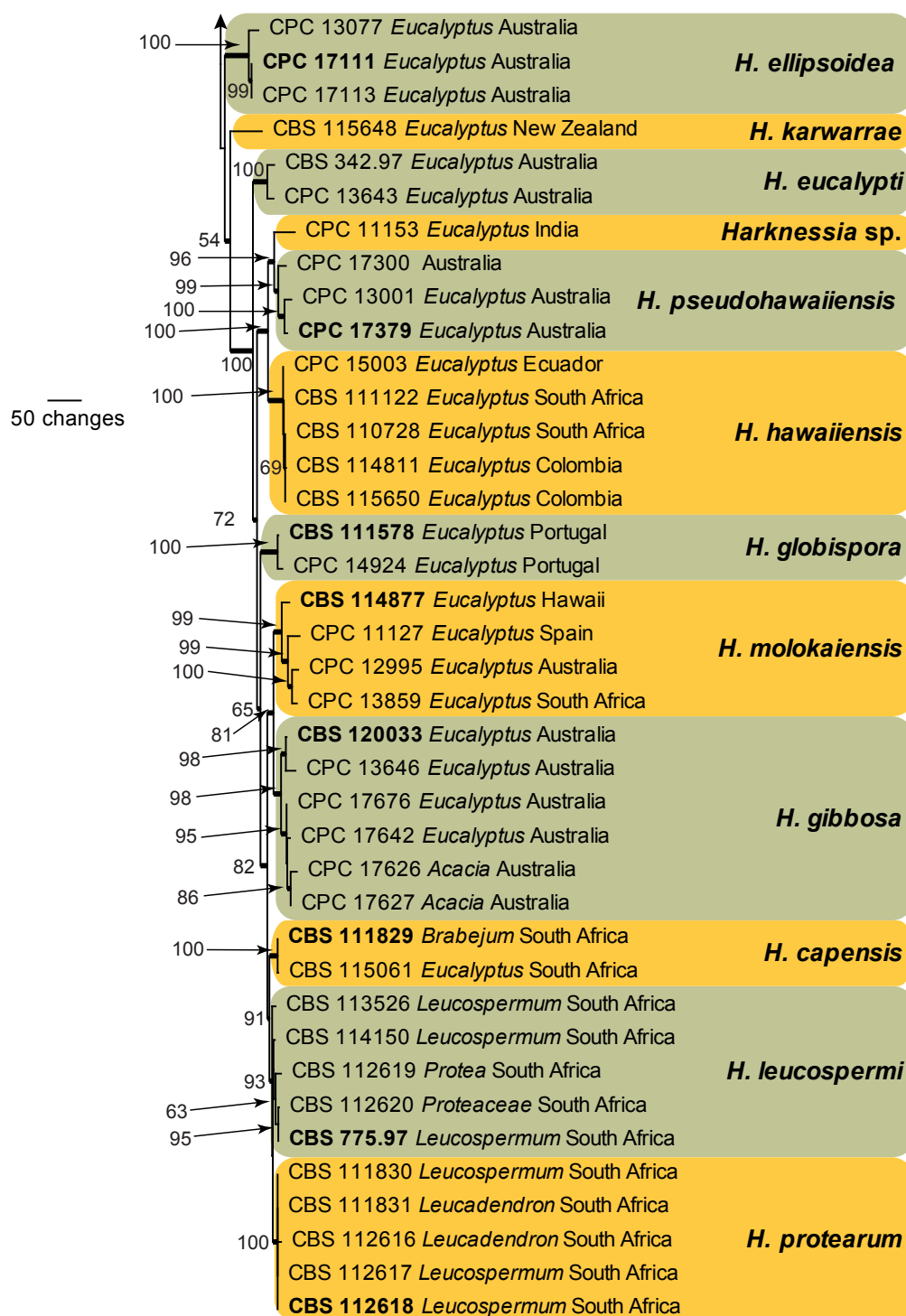
***Harknessia ravenstreetina*** Crous & R.G. Shivas, *sp. nov.* — MycoBank MB564745; Fig. 10

**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* pycnidoid, 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.

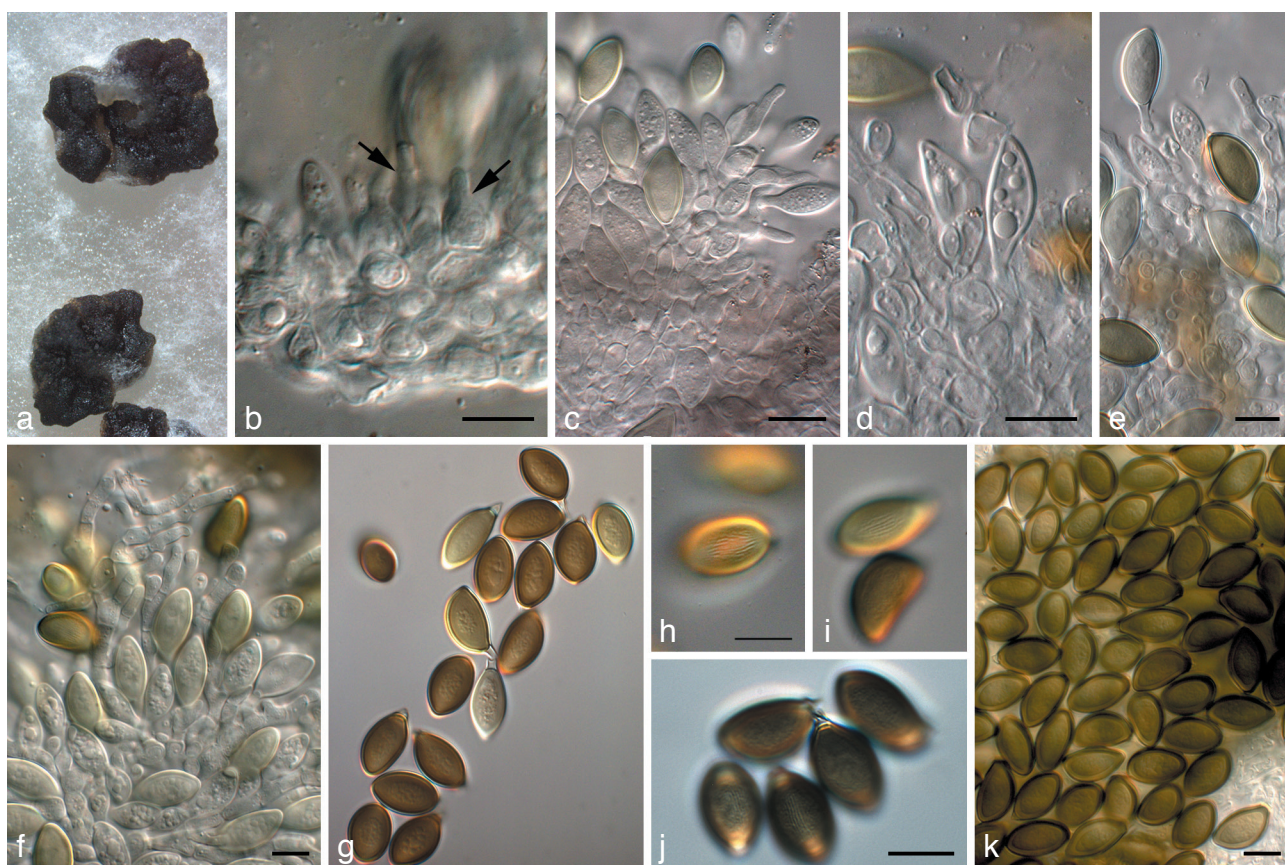
**Culture characteristics** — Colonies spreading, fluffy, with moderate to abundant aerial mycelium; surface dirty white to cream; cream in reverse; covering the dish in 1 mo.

**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.

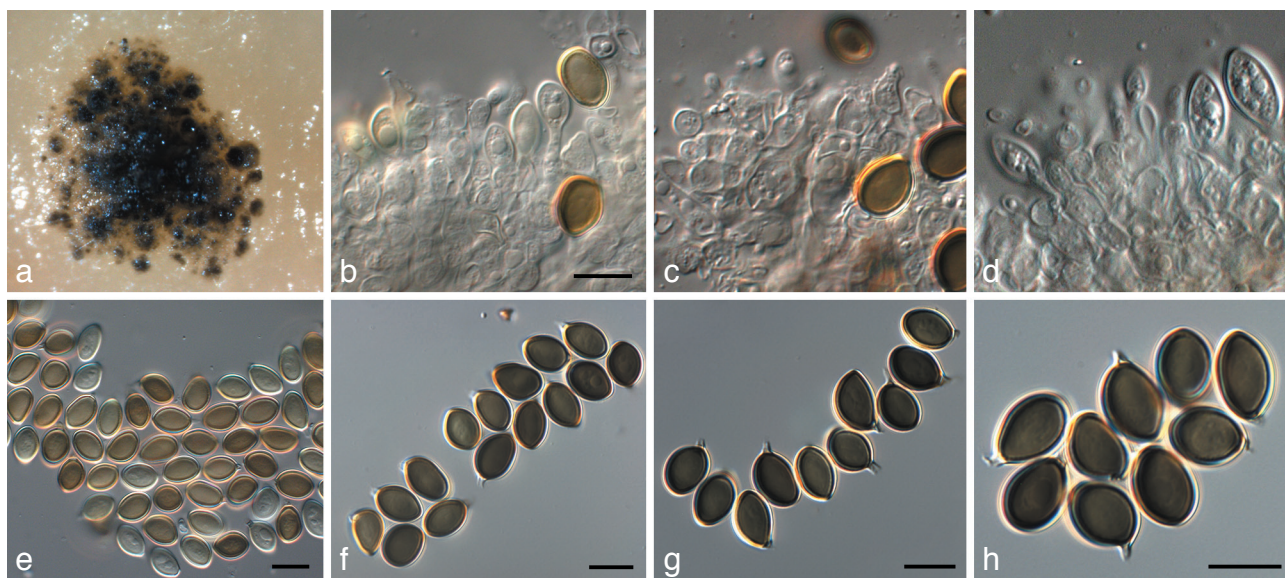
**Notes** — *Harknessia ravenstreetina* is similar to *H. antarctica* in conidium shape (conidia 20–24 × 10–12 µm, basal appendages 11–28 × 2–3 µm; Nag Raj 1993), although it has smaller conidia, and shorter basal appendages. Unfortunately, a culture of *H. antarctica* was not available for inclusion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

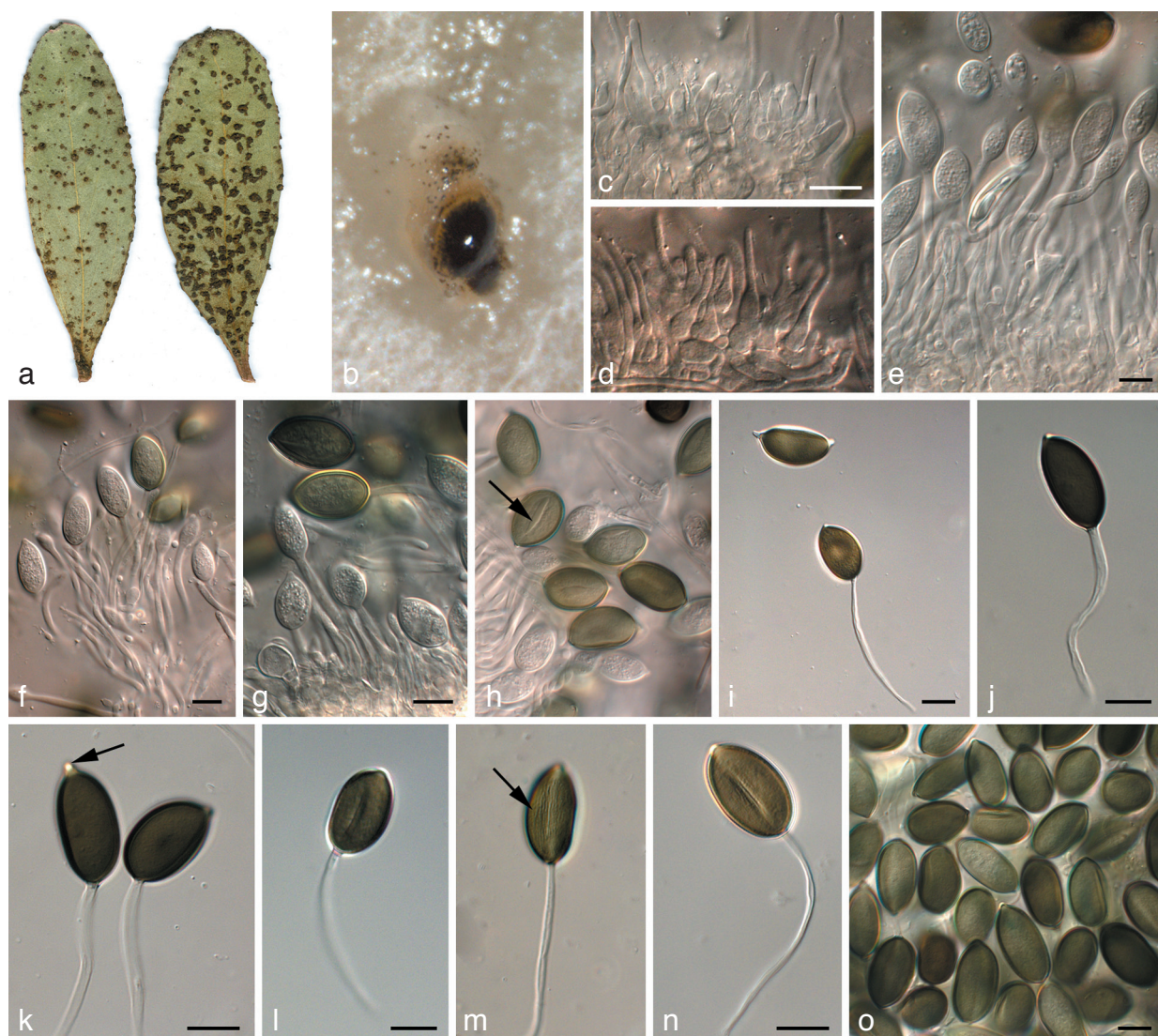
**Notes** — *Harknessia spermatoidea* was originally described from Spain, but the specimen collected on *Eucalyptus* from Cyprus closely matches the morphology observed in the holotype, enabling us to designate an epitype for this taxon. Although phylogenetically closely related to *H. ipereniae*, *H. 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

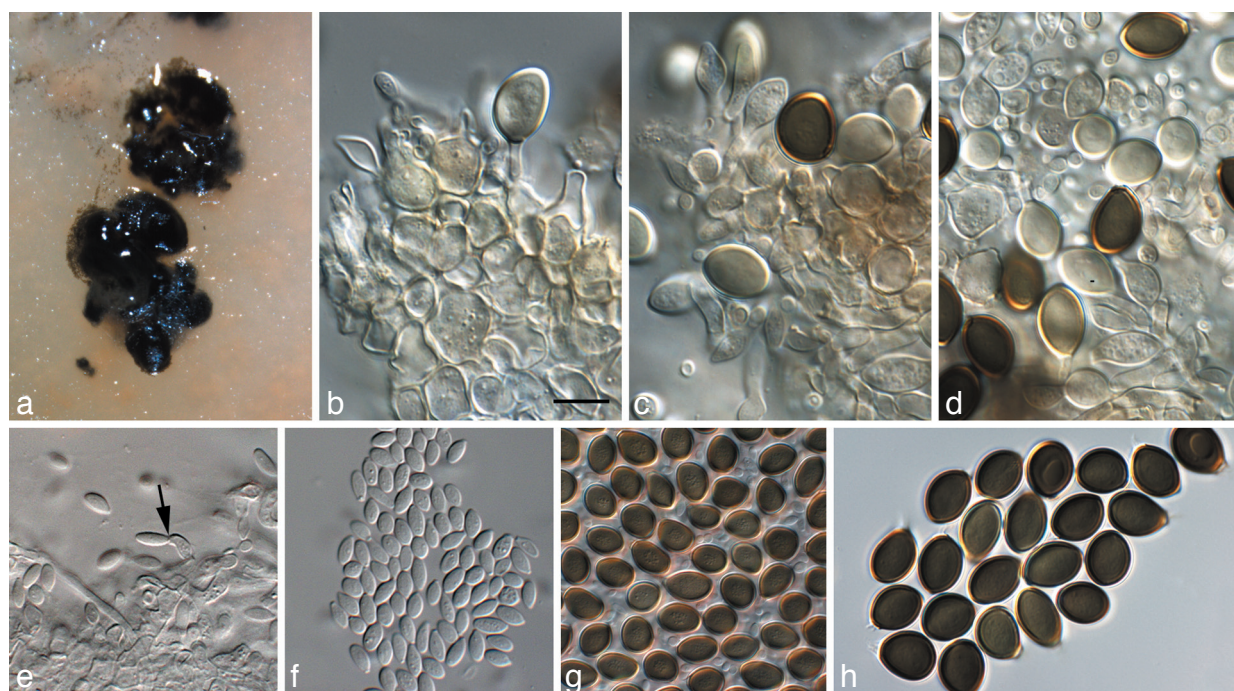
**Etymology.** Named after the location where it was collected in Italy, Viterbo.

**Foliicolous**, amphigenous, developing on brown leaf spots after incubation in moist chambers (presumed endophyte). Description on OA, as cultures remained sterile on PNA. **Conidiomata** pycnidiod, 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*



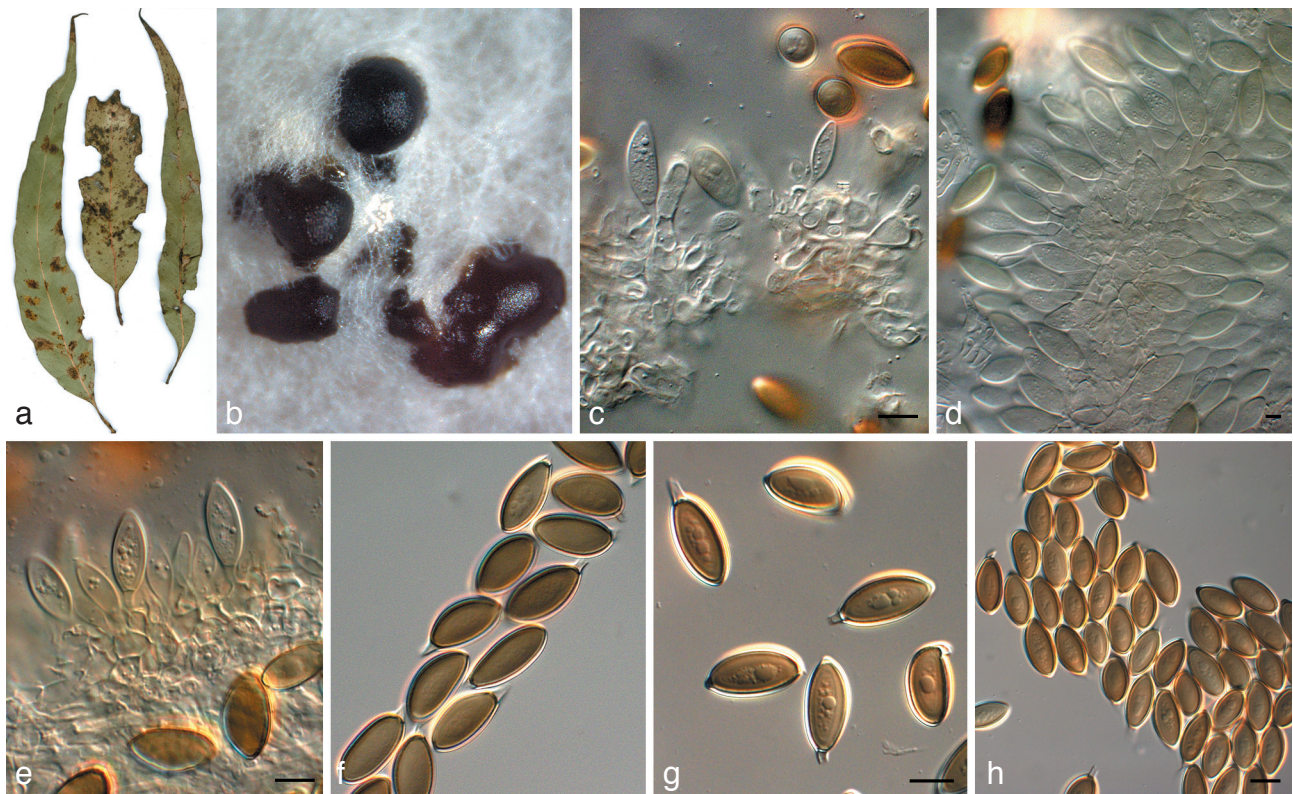


**Fig. 8** *Harknessia kleinzeeina* (CPC 16277). a. Insect damage on leaves, creating lesions from which *H. kleinzeeina* was isolated; b. sporulating colony on OA; c–g. conidiogenous cells giving rise to conidia; h–o. conidia with long basal appendages (arrow in k denotes apiculus, and in h and m longitudinal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

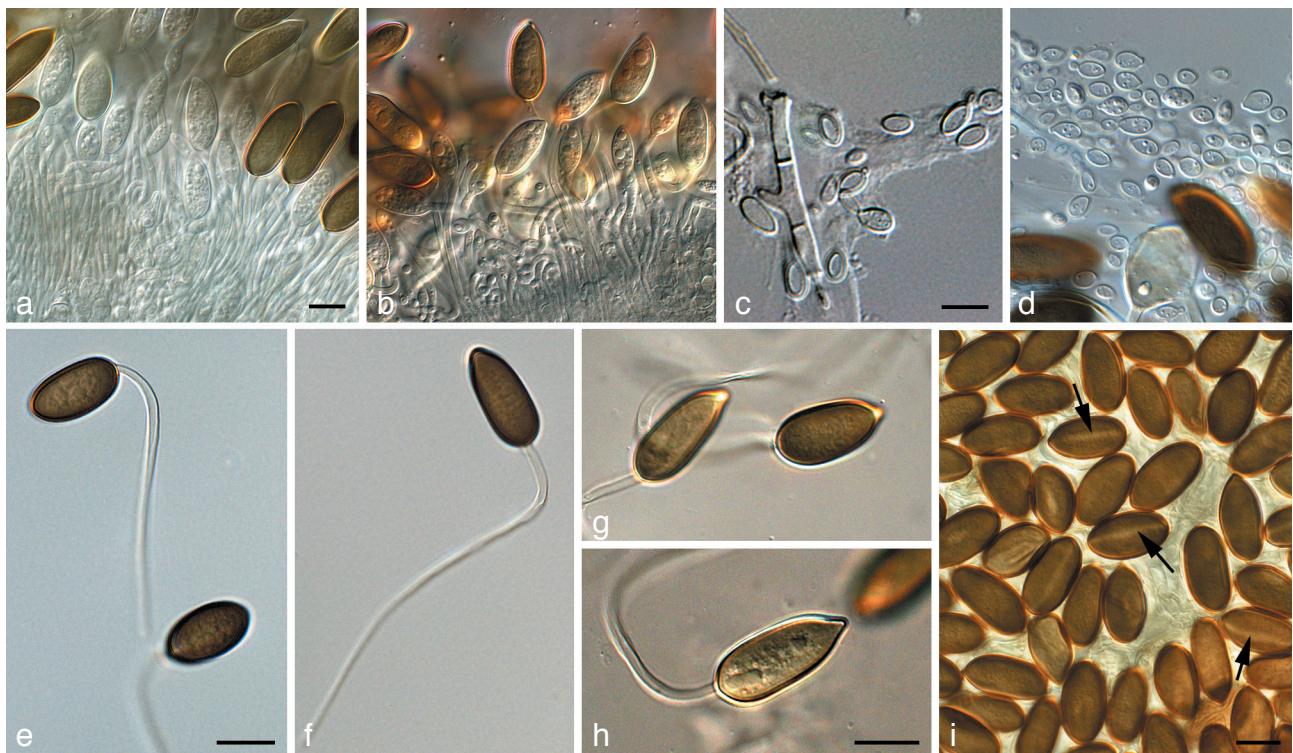


**Fig. 9** *Harknessia pseudohawaiiensis* (CPC 17380). a. Sporulating colony on OA; b–d. conidiogenous cells giving rise to conidia; e. microconidiogenous cell giving rise to microconidium (arrow); f. microconidia; g, h. macroconidia. — Scale bars = 10 µm.





**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,  $12\text{--}20 \times 4\text{--}6$  µm, becoming pale brown with age. Macroconidia  $(17\text{--})20\text{--}23\text{--}(25) \times (9\text{--})10\text{--}13\text{--}(15)$  µm (av.  $23 \times 12$  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  $(25\text{--})35\text{--}60 \times (2\text{--})3\text{--}(4)$  µm in vitro, hyaline, tubular, smooth, thin-walled, flexuous, devoid of cytoplasm, at times walls collapsing, covered in mucilaginous layer when im-



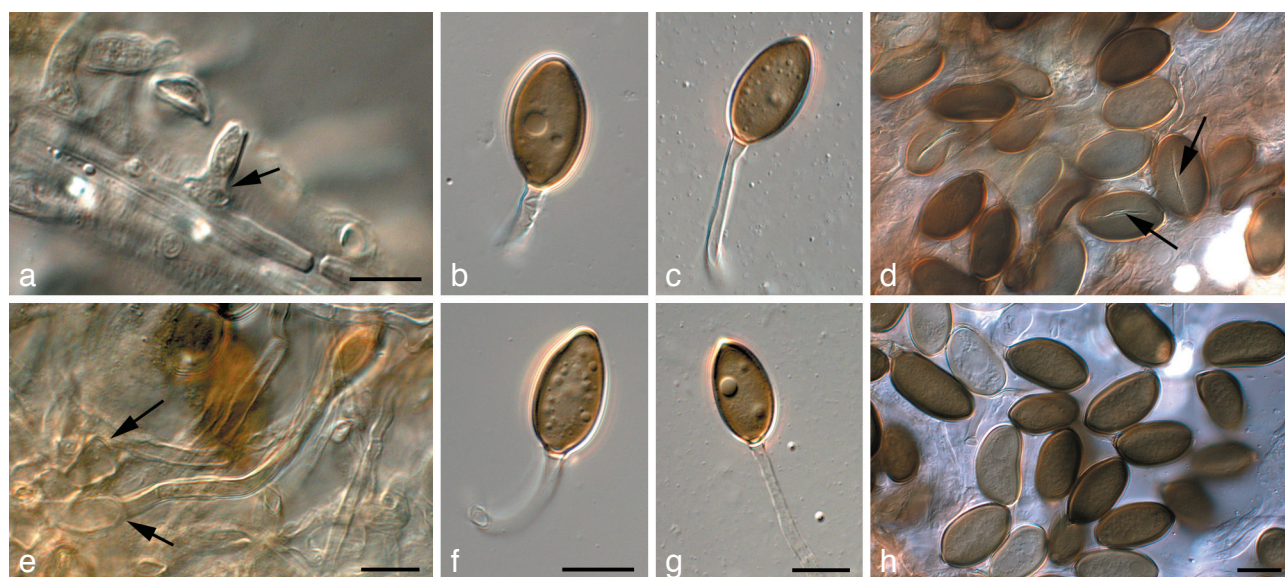


Fig. 12 *Harknessia viterboensis* (CBS 115647). a, e. Conidiogenous cells (arrows); b–d, f–h. conidia with appendages (arrows in d denote apparent germ slit). — Scale bars = 10 µm.

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. spermatodea*, *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,

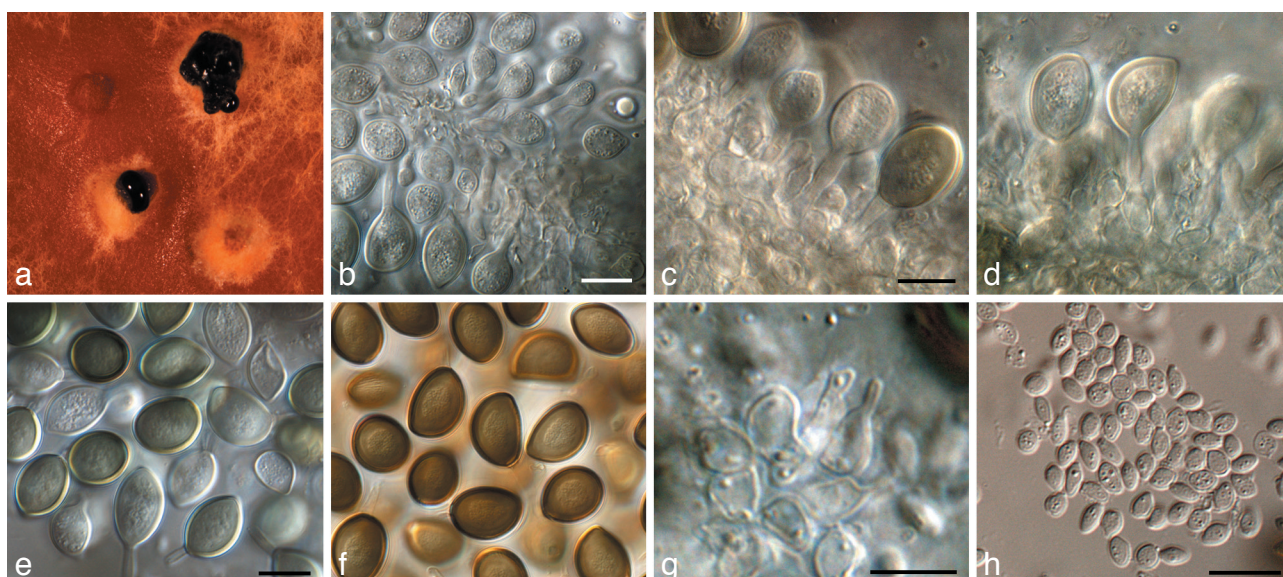
namely *Sydowiellaceae* (*Sydowiella* and aggregates), *Schizoparmeaceae* (*Schizoparme*/*Pilidiella* and *Coniella*; van Niekerk et al. 2004), *Gnomoniaceae* (more than 10 sexual genera; Mejía et al. 2011), *Cryphonectriaceae* (*Cryphonectria* generic complex; Gryzenhout et al. 2004, 2006), *Valsaceae* (*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, furfuraeous 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.

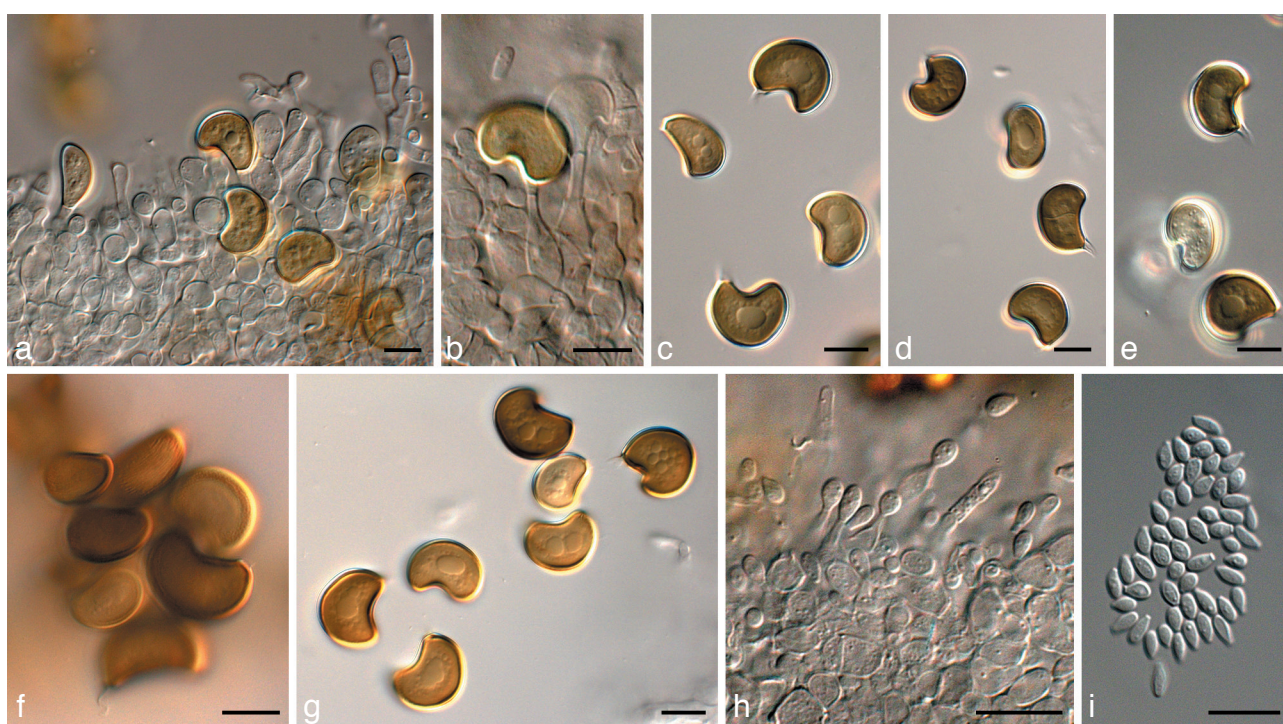
Although the genus *Harknessia* (type species *H. eucalypti*, teleomorph unknown) was recognised as a separate group in the *Diaporthales* (Castlebury et al. 2002), its family relationships remained unresolved. The main reason for this was that its teleomorph states were placed in *Wuestneia* (Crous 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 renisporea* (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 *Apharknessia* (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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## REFERENCES

- Adams GC, Wingfield MJ, Common R, Roux J. 2005. Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (Ascomycota, Diaporthales, Valsaceae) from Eucalyptus. *Studies in Mycology* 52: 1–142.
- Arx JA von, Müller E. 1954. Die Gattungen der amersporen Pyrenomycten. *Beiträge zur Kryptogamenflora der Schweiz* 11, 1: 1–434.
- Barr ME. 1978. Diaporthales in North America with emphasis on *Gnomonia* and its segregates. *Mycologia Memoir* 7: 1–232.
- Bettucci L, Saravay M. 1993. Endophytic fungi of *Eucalyptus globulus*: a preliminary study. *Mycological Research* 97: 679–682.
- Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556.
- Castlebury LA, Farr DF, Rossman AY, Jaklitsch WJ. 2003. *Diaporthe angeli* comb. nov., a modern description and placement of *Diaporthopsis* in *Diaporthe*. *Mycoscience* 44: 203–208.
- Castlebury LA, Rossman AY, Jaklitsch WJ, Vasileva LN. 2002. A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. *Mycologia* 94: 1017–1031.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* 50: 19–22.
- Crous PW, Knox-Davies PS, Wingfield MJ. 1989. Newly-recorded foliage fungi of *Eucalyptus* spp. in South Africa. *Phytophylactica* 21: 85–88.
- Crous PW, Mohammed C, Glen M, Verkley GJM, Groenewald JZ. 2007. *Eucalyptus* microfungi known from culture. 3. *Eucasphaeria* and *Symptenturia* genera nova, and new species of *Furcaspora*, *Harknessia*, *Heteroconium* and *Phacidia*. *Fungal Diversity* 25: 19–36.
- Crous PW, Rogers JD. 2001. *Wuestneia molokaiensis* and its anamorph *Harknessia molokaiensis* spp. nov. from *Eucalyptus*. *Sydowia* 53: 74–80.
- Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, et al. 2009a. Phylogenetic lineages in the Capnodiales. *Studies in Mycology* 64: 17–47.
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, et al. 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Studies in Mycology* 55: 235–253.
- Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds). 2009b. *Fungal Biodiversity. CBS Laboratory Manual Series 1: 1–269*. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.
- Crous PW, Wingfield MJ, Nag Raj TR. 1993. *Harknessia* species occurring in South Africa. *Mycologia* 85: 275–280.
- Crous PW, Wingfield MJ, Park RF. 1991. *Mycosphaerella nubulosa* a synonym of *M. molleriana*. *Mycological Research* 95: 628–632.
- Farr DF, Rossman AY. 2001. *Harknessia lythri*, a new species on purple loosestrife. *Mycologia* 93: 997–1001.
- Farr DF, Rossman AY. 2003. *Dwiwoopa*, a coelomycetous genus with two species. *Mycoscience* 44: 443–446.
- Glass NL, Donaldson G. 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323–1330.
- Gryzenhout M, Myburg H, Merwe NA van der, Wingfield BD, Wingfield MJ. 2004. *Crysoporthe*, a new genus to accommodate *Cryphonectria cubensis*. *Studies in Mycology* 50: 119–141.
- Gryzenhout M, Myburg H, Wingfield BD, Wingfield MJ. 2006. *Cryphonectriaceae* (Diaporthales) a new family including *Cryphonectria*, *Chrysosporthe*, *Endothia*, and allied genera. *Mycologia* 98: 239–249.
- Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, et al. 2011. The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* 2: 105–112.
- Höhnelt F von. 1914. Fragmente zur Mycologie 864. Über die Gattung *Harknessia* Cooke. *Sitzungsberichte der Akademie der Wissenschaften in Wien, Mathematisch – Naturwissenschaftliche Klasse. Abteilung 1*, 123: 86–87.
- Hoog GS de, Gerrits van den Ende AHG. 1998. Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* 41: 183–189.
- Lee S, Groenewald JZ, Crous PW. 2004. Phylogenetic reassessment of the coelomycete genus *Harknessia* and its teleomorph *Wuestneia* (Diaporthales), and the introduction of *Apharknessia* gen. nov. *Studies in Mycology* 50: 235–252.
- Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ. 2008. Microfungi occurring on Proteaceae in the fynbos. *CBS Biodiversity Series* 7: 1–166. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.
- Mejía LC, Castlebury LA, Rossman AY, Sogonov MV, White JF Jr. 2011. A systematic account of the genus *Plagiostoma* (Gnomoniaceae, Diaporthales) based on morphology, host-associations, and a four-gene phylogeny. *Studies in Mycology* 68: 211–235.
- Mostert L, Crous PW, Groenewald JZ, Gams W, Summerbell RC. 2003. *Togninia* (Calosphaeriales) is confirmed as teleomorph of *Phaeoacremonium* by means of morphology, sexual compatibility, and DNA phylogeny. *Mycologia* 95: 646–659.
- Mostert L, Crous PW, Kang J-C, Phillips AJL. 2001. Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. *Mycologia* 93: 145–166.
- Mostert L, Groenewald JZ, Summerbell RC, Gams W, Crous PW. 2006. Taxonomy and pathology of *Togninia* (Diaporthales) and its *Phaeoacremonium* anamorphs. *Studies in Mycology* 54: 1–115.
- Nag Raj TR. 1993. *Coelomycetous anamorphs with appendage-bearing conidia*. Mycologue Publications, Waterloo, Ontario.
- Nag Raj TR, DiCosmo F. 1981. A monograph of *Harknessia* and *Mastigosporella* with notes on associated teleomorphs. *Bibliotheca Mycologica* 80: 1–62.
- Niekerk JM van, Groenewald JZ, Verkley GJM, Fourie PH, Wingfield MJ, Crous PW. 2004. Systematic reappraisal of *Coniella* and *Piliidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. *Mycological Research* 108: 283–303.
- O'Donnell K, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7: 103–116.
- Park RF, Keane PJ, Wingfield MJ, Crous PW. 2000. Fungal diseases of eucalypt foliage. In: Keane PJ, Kile GA, Podger FD, Brown BN (eds). *Diseases and pathogens of eucalypts*: 153–239. CSIRO publishing, Australia.
- Quaedvlieg W, Kema GHJ, Groenewald JZ, Verkley GJM, Seifbarghi S, Razavi M, Mirzadi Gohari A, Mehrabi R, Crous PW. 2011. *Zymoseptoria* gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. *Persoonia* 26: 57–69.
- Rayner RW. 1970. A mycological colour chart. CMI and British Mycological Society. Kew, Surrey, England.
- Réblová M, Mostert L, Gams W, Crous PW. 2004. New genera in the Calosphaeriales: *Togninia* and its anamorph *Phaeoacrella*, and *Calosphaeriophora* as anamorph of *Calosphaeria*. *Studies in Mycology* 50: 533–550.
- Reid J, Booth C. 1989. On *Cryptosporella* and *Wuestneia*. *Canadian Journal of Botany* 67: 879–908.
- Rensburg JCJ van, Lamprecht SC, Groenewald JZ, Castlebury LA, Crous PW. 2006. Characterisation of *Phomopsis* spp. associated with die-back of rooibos (*Aspalathus linearis*) in South Africa. *Studies in Mycology* 55: 65–74.
- Rossman AY, Farr DF, Castlebury LA. 2007. A review of the phylogeny and biology of the Diaporthales. *Mycoscience* 48: 135–144.
- Sankaran KV, Sutton BC, Minter DW. 1995. A checklist of fungi recorded on *Eucalyptus*. *Mycological Papers* 170: 1–376.
- Summerell BA, Groenewald JZ, Carnegie AJ, Summerbell RC, Crous PW. 2006. *Eucalyptus* microfungi known from culture. 2. *Alysidiella*, *Fusculina* and *Phlogicylindrium* genera nova, with notes on some other poorly known taxa. *Fungal Diversity* 23: 323–350.
- Sutton BC. 1971. Coelomycetes. IV. The genus *Harknessia*, and similar fungi on *Eucalyptus*. *Mycological Papers* 123: 1–46.
- Sutton BC. 1980. The coelomycetes: Fungi imperfecti with pycnidia, acervuli, and stromata. Commonwealth Mycological Institute, Kew, Surrey.
- Sutton BC, Pascoe I. 1989. Addenda to *Harknessia* (Coelomycetes). *Mycological Research* 92: 431–439.
- Swart L, Crous PW, Denman S, Palm ME. 1998. Fungi occurring on Proteaceae I. *South African Journal of Botany* 64: 137–145.
- Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.
- White TJ, Bruns T, Lee J, Taylor SB. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR protocols: a guide to methods and applications*: 315–322. Academic Press, San Diego, California, USA.
- Wingfield MJ, Beer ZW de, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous PW. 2012. One fungus, one name promotes progressive plant pathology. *Molecular Plant Pathology* doi: 10.1111/J.1364-3703.2011.00768.X.
- Yuan ZQ, Mohammed C. 1997. *Wuestneia epispora* sp. nov. on stems of eucalypts from Australia. *Mycological Research* 101: 195–200.
- Zhang N, Blackwell M. 2001. Molecular phylogeny of dogwood anthracnose fungus (*Discula destructiva*) and the Diaporthales. *Mycologia* 93: 355–365.