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 33 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 theolytic ascospores. Six species occurring on Eucalyptus are newly introduced, namely H. australiensis, H. ellipsoides, H. pseudohawaiiensis, and H. ravenstreetina from South Africa, and H. viterboensis from Italy. Epitypes are designated for H. spermatoidea and H. waresubiae, 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.

Abstract  Harknessiaceae is introduced as a new family in the ascomycete order Diaporthales to accommodate species of Harknessiaceae 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 theolytic ascospores. Six species occurring on Eucalyptus are newly introduced, namely H. australiensis, H. ellipsoides, H. pseudohawaiiensis, and H. ravenstreetina from South Africa, and H. viterboensis from Italy. Epitypes are designated for H. spermatoidea and H. waresubiae, 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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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-
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. asc; m. ascospores; n. conidiomata oozing conidia; o–q. conidia with basal appendages and central guttules. — Scale bars = 10 µm.
| Species                  | Culture accession numbers | Substrate                                      | Country          | Collector        | GenBank accession numbers |
|--------------------------|---------------------------|------------------------------------------------|------------------|------------------|--------------------------|
| Apoharknessia insueta    | CPC 10947; CBS 114575     | Leaf spots on Eucalyptus sp.                   | Colombia         | M.J. Wingfield   | JQ706082 – – – AY720813  |
|                         | CPC 11175                 | Yucca elephantipes                             | Costa Rica       | A. Igram         | JQ706209                 |
|                         | CPC 1451; CBS 111377*     | Leaves of Eucalyptus pellita                  | Brazil           | P.W. Crous       | JQ706083 – – – AY720814  |
| Foliocyphia eucalypti   | CPC 12494; CBS 124779*    | Eucalyptus coccofera                          | Australia: Western Cape Province | C. Mohammed  | JQ706128 – GQ303307     |
| Harknessia australiensis| CPC 13596; CBS 132120     | Leaves of Eucalyptus sclerophylla            | Australia: New South Wales | B.A. Summerrill | JQ706129 JQ706170 JQ706120 |
|                         | CPC 15029*; CBS 132119    | Leaves of Eucalyptus disstria                 | Australia: New South Wales | B.A. Summerrill | JQ706130 JQ706171 JQ706121 |
| Harknessia capensis      | CPC 10876; CBS 115061     | Eucalyptus leaves                             | South Africa: Western Cape Province | P.W. Crous | AY720718 AY720750 AY720815 |
|                         | CPC 5468; CBS 111829*     | Dead twigs and leaf litter of Brabejum stellatifoliun | South Africa: Western Cape Province | S. Lee | AY720719 AY720751 AY720816 |
| Harknessia ellipsoidea   | CPC 13077; CBS 132122     | Leaves of Eucalyptus propinque                | Australia: New South Wales | B.A. Summerrill | JQ706131 JQ706172 JQ706122 |
|                         | CPC 17111*; CBS 132121    | Leaves of Eucalyptus sp.                      | Australia: Queensland | P.W. Crous & R.G. Shivas | JQ706132 JQ706173 JQ706123 |
|                         | CPC 17113*                | Leaves of Eucalyptus sp.                      | Australia: Queensland | P.W. Crous & R.G. Shivas | JQ706133 JQ706174 JQ706124 |
| Harknessia eucalyptii    | CBS 342.97                | Eucalyptus regnans                            | Australia: Tasmania | Z.-Q. Yuan       | AY720745 AY720777 AY20808 AF408363 |
|                         | CPC 13643                 | Eucalyptus regnans                            | Australia: Tasmania | B.A. Summerrill | JQ706089 JQ706134 JQ706175 JQ706125 |
| Harknessia eucalyptorum  | CBS 113620                | Leaves of Eucalyptus sp.                      | Spain            | P.W. Crous & G. Bills | AY720746 AY720778 AY20809 AY720839 |
|                         | CPC 85; CBS 111115*       | Leaves of Eucalyptus andreawali              | South Africa: Western Cape Province | P.W. Crous | AY720747 AY720779 AY20810 AY720840 |
|                         | CPC 11302                 | Eucalyptus sp.                                | Italy            | W. Gams         | JQ706135 JQ60176 –        |
|                         | CPC 12697                 | Leaf litter of Eucalyptus sp.                 | South Africa: Western Cape Province | P.W. Crous | JQ706091 JQ706136 JQ60177 JQ706126 |
|                         | CPC 13074                 | –                                              | Italy            | W. Gams         | JQ706092 JQ706137 –        |
|                         | CPC 14951                 | Eucalyptus sp.                                | Portugal         | P.W. Crous       | JQ706093 JQ706138 JQ60178 JQ706128 |
|                         | CPC 14954                 | Eucalyptus sp.                                | Portugal         | P.W. Crous       | JQ706094 – – – JQ706129   |
| Harknessia fusiformis    | CPC 295; CBS 110785*      | Leaf litter of Eucalyptus sp.                 | South Africa: Orange Free State | P.W. Crous | AY720721 AY720753 AY720818 |
|                         | CPC 10488; CBS 115649     | Leaves of Eucalyptus sp.                      | South Africa: Orange Free State | P.W. Crous | AY720720 AY720752 AY720817 |
|                         | CPC 11124                 | Eucalyptus sp.                                | New Zealand      | J. Stalpers      | JQ706096 JQ706139 JQ60179 –        |
|                         | CPC 13649                 | Eucalyptus globulus                           | Australia: Tasmania | B.A. Summerrill | JQ706097 JQ706140 JQ60180 JQ706120 |
|                         | CPC 16550                 | Eucalyptus dyes                               | Australia: Southern Highlands | B.A. Summerrill | JQ706098 JQ706141 JQ60181 JQ706121 |
| Harknessia gibbosa       | CPC 12473; CBS 120033*    | Eucalyptus delegatensis                       | Australia: Tasmania | C. Mohammed     | EF110615 JQ706142 JQ60182 EF110615 |
|                         | CPC 13646                 | Eucalyptus delegatensis                       | Australia: Tasmania | B.A. Summerrill | JQ706099 JQ706143 JQ60183 JQ706122 |
|                         | CPC 17626                 | Acacia pyrana                               | Australia: Victoria | P.W. Crous | JQ706100 JQ706144 JQ60184 JQ706123 |
|                         | CPC 17627                 | Acacia pyrana                               | Australia: Victoria | P.W. Crous | JQ706101 JQ706145 JQ60185 JQ706124 |
|                         | CPC 17642                 | Eucalyptus sp.                               | Australia: Victoria | P.W. Crous | JQ706102 JQ706146 JQ60186 JQ706125 |
|                         | CPC 17676                 | Eucalyptus sp.                               | Australia: Victoria | P.W. Crous | JQ706103 JQ706147 JQ60187 JQ706126 |
| Harknessia globispora    | CPC 12799                 | Eucalyptus globulus                           | Portugal         | A.L. Phillips    | JQ706104 – JQ706188 JQ706127 |
|                         | CPC 14924                 | Eucalyptus sp.                               | Portugal         | P.W. Crous       | JQ706105 JQ706148 JQ706189 JQ706128 |
|                         | CPC 3710; CBS 111578*     | Leaf litter of Eucalyptus globulus            | Portugal         | S. Denman        | AY720722 AY720754 AY720785 AY720819 |
| Harknessia hawaiiensis   | CPC 10976; 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 JQ60190 JQ706129 |
|                         | CPC 113; CBS 110728       | Leaves of Eucalyptus viminata                | South Africa: Western Cape Province | P.W. Crous | AY720725 AY720757 AY720788 AY720822 |
|                         | CPC 15003                 | Eucalyptus sp.                               | Ecuador          | A.C. Allenas     | 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*    | Eucalyptus leaf litter                       | Australia: Western Australia | A. van Iperen  | EF110614 JQ706151 JQ60192 EF110614 |
| Harknessia karwarrae     | CPC 10928; CBS 115648     | Leaves of Eucalyptus botryoides              | New Zealand      | M. Dick          | AY720748 AY720780 AY208011 AY720841 |
| Harknessia kleinzeina | CPC 108; CBS 110729 | Eucalyptus leaf litter | South Africa: Western Cape Province | P.W. Crous | AY720739 AY720771 AY720802 – |
|----------------------|--------------------|-----------------------|--------------------------------------|------------|------------------|
| Harknessia leucospermi | CPC 16273 | Leaves of Eucalyptus sp. | South Africa: Northern Cape Province | Z.A. Pretorius | JQ706108 JQ706152 JQ706193 JQ706231 |
| Harknessia protea | CPC 1373; CBS 775.97ET | 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 115326 | Dead twigs of Leucospermum paecox | South Africa: Western Cape Province | S. Lee | AY720729 AY720761 AY720792 AY720826 |
| | CPC 5403; CBS 112620 | Dead twigs of unidentifed 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 112618ET | 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 5470; CBS 111831 | Dead twigs of unidentified tree (Proteaceae) | South Africa: Western Cape Province | S. Lee | AY720735 AY720767 AY720798 AY720831 |
| | CPC 5469; CBS 111830 | Dead twigs of Leucospermum sp. | South Africa: Western Cape Province | S. Lee | AY720736 AY720768 AY720799 AY720832 |
| Harknessia renispora | CBS 153.71ET | Dead leaf of Melaleuca pubescens | Australia: Victoria | H.J. Swart | AY720737 AY720769 AY720800 AY720833 |
| Harknessia rhadophthora | CPC 17163 | Callistemon prinulifolius | Australia: Queensland | P.W. Crous | JQ706114 JQ706158 JQ706199 JQ706237 |
| Harknessia salmonicola | CPC 12455; CBS 122372 | Eucalyptus nitida | Australia: Tasmania | M. Glen | JQ706115 JQ706159 – JQ706238 |
| | CPC 12922; CBS 120082ET | Leaves of Corymbia henryi | Australia: New South Wales | B.A. Summerell | DQ923532 – – DQ923532 |
| Harknessia renispora | CPC 13001 | Leaves of Eucalyptus tereicornis | Australia: Queensland | P.W. Crous | JQ706110 JQ706154 JQ706195 JQ706233 |
| | CPC 17300 | Leaves of Eucalyptus sp. | Australia: Queensland | P.W. Crous | JQ706111 JQ706155 JQ706196 JQ706234 |
| Harknessia renispora | CPC 17379; CBS 132124 | Leaves of Eucalyptus dunnii | Australia: New South Wales | A. Carnegie | JQ706111 JQ706155 JQ706196 JQ706234 |
| Harknessia reinigmatina | CPC 17085; 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 erinacea | CBS 11153 | Leaf litter of Eucalyptus sp. | India | W. Gams | JQ706119 JQ706162 JQ706202 – |
| Harknessia spermatoidea | CPC 13397; CBS 132127 | Leaf litter of Eucalyptus sp. | Cyprus | A. van Iperen | JQ706120 JQ706163 JQ706203 JQ706241 |
| Harknessia syzygi | CPC 184; CBS 111124ET | Syzygium cordatum | South Africa: Limpopo | M.J. Wingfield | AY720738 AY720770 AY720801 AY720834 |
| Harknessia vittorboensis | CPC 10843; CBS 11564ET | Leaves of Eucalyptus sp. | Italy | W. Gams | AY720740 AY720772 AY720803 AY720842 |
| Harknessia weberiae | CPC 12718; CBS 132129 | Eucalyptus sp. | South Africa: Western Cape Province | P.W. Crous | JQ706121 JQ706164 JQ706204 JQ706243 |
| | CPC 17670; CBS 132128 | Eucalyptus leaf litter | Australia: Victoria | P.W. Crous, J. Edwards, | 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 11487 | Eucalyptus robusta | USA: Hawaii | J.D. Rogers | AY720749 AY593935 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.
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. 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 nested 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, Schizophacmae, and Harknessia (MrBayes). From the analyses, it is evident that Cryphonectriaceae, Schizophacmae 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 Schizophacmae 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 Schizophacmae, 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

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.
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 1829 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 1000 equally most parsimonious trees were saved (TL = 1813 steps; CI = 0.673; RI = 0.850; RC = 0.572). While many species clades are well-defined, the interspecific variation for some species such as *H. fusiformis* and *H. renispora* is large, resulting in less support for these clades. Only the first 1000 equally most parsimonious trees with similar topologies to those of the parsimony analysis were obtained for ITS, CAL, and TUB, respectively, of the isolates of *Wuestneia* (presumed endophyte). *Conidiomata* pycnidioideum, stromatic, ampigenous, 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 conidigenous 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) × 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.

**Notes** — The *Harknessiaceae* is based on *C. hypodermia* and *C. dissita* and 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* pycnidioideum, stromatic, ampigenous, 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 conidigenous 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) × 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 ellipsoida** 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* pycnidioideum, stromatic, ampigenous, 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 conidigenous 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) × 2 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; 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-hawaiensis but differ by being broadly ellipsoidal in shape.
Harknessia kleinzeeina Crous, sp. nov. — MycoBank MB564743; Fig. 8

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

Folicolous, associated with irregular leaf spots induced by insect damage, pale brown, but appearing to be secondary infections, probably saprobic. Description on PNA. Conidiomata pycnidiod, subepidermal, becoming erumpent, aroid, black, up to 350 µm diam; dehiscence irregular with wide opening and border with pale yellow furfuraceous cells; wall of brown textura angularis. Conidiophores reduced to conidiogenous cells lining the base of conidomatal cavity. Conidioiogenous 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 pseudohawaiensis Crous & Carnegie, sp. nov. — MycoBank MB564744; Fig. 9

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

Folicolous, isolated from leaves incubated in moist chambers (presumed endophyte). Conidiomata pycnidiod, 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...
conidiomatal cavity. *Microconidiogenous cells* 5–9 × 4–6 μm, ampulliform to lageniform, hyaline, smooth, invested in mucilage, proliferating several times percurrently near apex. *Conidiophores* 11–15 × 6.5–8 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. *Microconidium* 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, Dundarabbin, Neaves plantation, S30°10′15″E152°30′33″, on leaves of *Eucalyptus dunnii*, CBS H-20514 holotype, cultures ex-type CPC 17380, 17379= CPC 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 pseudohawaiensis* is similar to *H. hawaiensis* in macroconidial shape, the presence of longitudinal striations, and the abundance of microconidia. It differs in having smaller macroconidia than *H. hawaiensis* (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-

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**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.
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-29924 epitype designated here, culture ex-epitype CPC 13937 = CBS 132127. — Spain, Pontavedra, La Toja, on leaf litter of Eucalyptus globulus, 4 Oct. 1985, N. Marzano, GM-RG 9320 (holotype), IMI 295508 (isotype).
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 pseudohawaiensis (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.
**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.

**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-
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. spermatioides*, *H. ipericinia* 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 10843 = CBS 113073 = CPC 5108; Western Cape Province, Malmesbury, on leaf litter of *Eucalyptus* sp., 22 Sept. 1979, B. Kendrick, DAOM 173902; 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 10843 = 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*/Pliidiella* and **Coniella**; van Niekerk et al. 2004), **Gnomoniaceae** (more than 10 sexual genera; Mejia et al. 2011), **Cryphonectriaceae** (**Cryphonectria** generic complex; Gryzenhout et al. 2004, 2006), **Valsaaceae** (**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**), **Pseudovalsaeeae** (**Pseudovalsa**; Castlebury et al. 2002), and **Togniniaeae** (**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**, teleorphem 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 *Aphoharknessia* (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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