Resolving *Tiarosporella* spp. allied to Botryosphaeriaceae and Phacidiaceae

PEDRO W. CROUS1, 2, 3, MICHAEL M. MÜLLER4, ROMINA M. SÁNCHEZ5, LUCRECIA GIORDANO5, M. VIRGINIA BIANCHINOTTI5, FREDA E. ANDERSON5 & JOHANNES Z. GROENEWALD1

1CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: p.crous@cbs.knaw.nl
2Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa
3Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
4Natural Resources Institute Finland (LUKE), P.O. Box 18, FIN-01301 Vantaa, Finland
5Centro de Recursos Naturales Renovables de la Zona Semiárida-Universidad Nacional del Sur Camino La Carrindanga Km 7, B8000FWB, Bahía Blanca, Argentina

Abstract

The type species of the genus *Tiarosporella*, *T. paludosa*, is epitypified and confirmed as a member of the Botryosphaeriaceae. Based on morphology and DNA sequence data of the large subunit nuclear ribosomal RNA gene (LSU, 28S) and the internal transcribed spacers (ITS) and 5.8S rRNA gene of the nrdNa operon, the genus *Tiarosporella* is shown to be poly- and paraphyletic. A group of isolates morphologically similar to *T. paludosa* cluster to the Phacidiaceae (Phacidiales, leotiomycetes) and we accommodated them in *Darkera*, a genus associated with needle diseases of conifers, with *D. picea* introduced as a novel taxon. This new taxon includes isolates occurring on needles of *Picea* spp. in Europe (Finland, Norway and Switzerland) and differs from *D. parca* according to a five-locus alignment consisting of ITS, LSU, partial 18S nuclear ribosomal rRNA, translation elongation factor 1-alpha and beta-tubulin genes. Four novel genera are introduced for tiarosporella-like fungi, namely *Eutiarosporella* based on *E. tritici* on *Triticum aestivum* from South Africa, *Marasasiomyces* based on *M. karoo* on *Eriocephalus* sp. from South Africa, *Mucoharknessia* based on *M. cortaderiae* on *Cortaderia selloana* from Argentina, and *Sakireeta* based on *S. madreeya* on *Aristida setacea* from India. Together with the genus *Botryobambusa*, these genera represent a subclade in the Botryosphaeriaceae that is ecologically diverse, occurring on *Poaceae*, as well as woody hosts, including endophytes, saprobes, and plant pathogens.

Keywords: coelomycetes, Dothideomycetes, ITS, LSU, Phacidiaceae, systematics

Introduction

Several coelomycetous genera with appended, hyaline conidia are members of the Botryosphaeriaceae, namely *Phylllosticta* (Phylllostictaceae; Wikee et al. 2013), *Melanops* (Melanopsaceae; Slippers et al. 2013), *Kellermania* (Planistromellaceae; Minnis et al. 2012), *Macrophomina* (Sarr et al. 2014), *Alanphillipsia*, *Botryobambusa*, *Botryosphaeria* and *Pseudofusicoccum* (Botryosphaeriaceae; Crous et al. 2006, 2013, Liu et al. 2012, Phillips et al. 2013). Many other genera also belong to the Botryosphaeriaceae, e.g. *Tiarosporella* (Crous et al. 2006), but due to a lack of cultures and DNA data, these connections have largely remained unconfirmed.

A genus allied to *Tiarosporella* is *Neottiospora*, based on *N. caricina* (Desmazières 1843), which was introduced for coelomycetes with pycnidial conidiomata, phialidic conidiogenous cells, and hyaline, unicellular conidia with evanescent mucoid appendages (Nag Raj 1973). In a re-examination of type material by Subramanian & Ramakrishnan (1957), they observed *Neottiospora* to have a conidial appendage, and considered it similar to the genus *Tiarosporella*, which was introduced by Von Höhnel (1919), based on *T. paludosa*. The appendage in *Neottiospora* was, however, shown to be basal by Nag Raj (1993), in contrast to the apical appendage observed in *Tiarosporella*. The genus *Tiarosporella*, based on *T. perforans*, is again distinguished from these genera by having 1-septate conidia, with bipolar appendages (Nag Raj 1993). Subramanian & Ramakrishnan (1957) also introduced the genus *Sakireeta*, based on *S. madreeya*, which has plurilocular conidiomata formed in a stroma. Furthermore, Subramanian (1961) introduced the
genus *Neottiosporina*, based on *N. apoda*, for a pycnidial coelomycete with appendaged, 3-septate, pigmented conidia. In their treatment of the genus, Sutton & Alcorn (1974) considered conidia of *N. apoda* to be hyaline, and thus also described *N. masonii* in the genus. Nag Raj (1993) did not consider conidial pigmentation of paramount importance in this genus, and hence also allocated several species with hyaline conidia to it, the unifying factor being that the conidia were septate, unlike the aseptate conidia of *Tiarosporella*.

Species of *Tiarosporella* have traditionally been associated with members of *Poaceae* (Sutton & Marasas 1976, Nag Raj 1993), although recent studies have also reported them from woody hosts (Jami *et al.* 2012, 2014). Not much is known about the pathogenicity of these fungi, but several species of *Tiarosporella* have been associated with needle diseases of conifers, either as pathogens or endophytes (Sieber 1988, Karadžić 1998, Müller & Hantula 1998), some of which have been linked to sexual morphs in *Darkera* in Phacidiaceae (Phaciidales, Leotiomycetes) (Whitney *et al.* 1975, DiCosmo *et al.* 1984). Species of *Phacidium s.str.* (Phaciidae) have been shown to cluster with *Ceuthospora* asexual morphs, which also have hyaline conidia with apical mucoid appendages (Crous *et al.* 2014). The relation of *Tiarosporella* species included in the Botryosphaeriaceae, to other similar morphs included in the Phaciidaeae has so far remained unclear. The aim of the present study was thus to resolve the generic relationships of this complex as far as possible, and delineate those genera for which cultures could be obtained.

**Materials and Methods**

**Isolates**

Tissue samples showing conidiomata were placed in moist chambers to enhance sporulation. Single conidial colonies were grown in Petri dishes containing 2% malt extract agar (MEA) as described earlier (Crous *et al.* 1991). Colonies were subcultured onto potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al.* 2009b), and pine needle agar (PNA) (Smith *et al.* 1996), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains were deposited at the CBS-KNAW Fungal Biodiversity Centre in Utrecht, Netherlands (CBS).

**DNA isolation, amplification and analyses**

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) following the manufacturer’s protocols. Part of the nuclear rDNA operon spanning the 3’ end of the 18S nrRNA gene, both internal transcribed spacer regions, the 5.8S nrRNA gene, and the first approximately 950 nucleotides of the 5’ end of the 28S nrRNA gene (ITS) was amplified using the primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990). The primers ITS4 (White *et al.* 1990) and LSU1Fd (Crous *et al.* 2009a) were used as internal sequence primers to provide sequences of high quality over the entire length of the amplicon. Part of the 5’ end of the 18S nrRNA gene was amplified and sequenced with the primers NS1 and NS4 (White *et al.* 1990), part of the translation elongation factor 1-alpha gene (TEF) with the primers EFl-728F (Carbone & Kohn 1999) and EF-2 (O’Donnell *et al.* 1998) and part of the beta-tubulin gene using primers TUB3Fd and TUB4Rd (Groenewald *et al.* 2013) or Bt-2a and Bt-2b (Glass & Donaldson 1995). The sequence alignment and subsequent phylogenetic analyses were carried out using methods described by Lombard *et al.* (2011); gaps were treated as “fifth state” data. The alignment for the Botryosphaeriaceae is based on the dataset used by Phillips *et al.* (2013). Sequences derived in this study were lodged in GenBank (Table 1) and the alignments in TreeBASE (www.treebase.org/treebase/index.html).

**Morphology**

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. Measurements and photographs were made from structures mounted in clear lactic acid. The 95% confidence intervals were derived from 30 observations (× 1000 magnification), with the extremes given in parentheses. Ranges of the dimensions of other characters are given. Colony colours (surface and reverse) were established using the colour charts of Rayner (1970). Recently collected sections of leaves bearing fruiting bodies of the fungus were pressed, and preserved in the Herbarium of the Biology Department, Universidad Nacional del Sur (BBB), or at the CBS in Utrecht, and taxonomic novelties were deposited in MycoBank (Crous *et al.* 2004).
<table>
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<td>CBS 448.91 ex-type</td>
<td>Dead culms of <em>Bambusa arundinacea</em></td>
<td>Sierra Leone</td>
<td>F.C. Deighton</td>
<td>KF766199 DQ377866</td>
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<td>CBS 145.78 ex-isotype</td>
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<td>Sophora chrysophylla</td>
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<td>ICMP 16812 ex-type</td>
<td>Recently dead bark-covered twigs of Citrus sinensis</td>
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<td>S.R. Pennycook, P.R. Johnston &amp; B.C. Paulus</td>
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<td>CBS 133993; MFLUCC 11-0579 ex-type</td>
<td>Dead twig of Eucalyptus sp.</td>
<td>Thailand: Chiang Rai Province</td>
<td>M. Dolom</td>
<td>JX646802 JX646819</td>
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<td>Eleocharis palustris</td>
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<td>K. &amp; L. Holm</td>
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<td>Leaf spot on Xanthorrhoea sp.</td>
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1 CAP: A.J.L Phillips, Universidade Nova de Lisboa, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC: Culture collection of Pedro Crous, housed at CBS; ICMP: International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; LYN: Private culture collection Frank Hill, New Zealand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Mai, Thailand; UAMH: University of Alberta Mold Herbarium and Culture Collection, Edmonton, Canada; UPSC: Uppsala University Culture Collection of Fungi, Botanical Museum University of Uppsala, Uppsala, Sweden; WAC: Department of Agriculture, Western Australia Plant Pathogen Collection, South Perth, Western Australia.
2 ITS: internal transcribed spacers and intervening 5.8S rDNA; LSU: large subunit (28S) of the rRNA gene operon; TEF: partial translation elongation factor 1-alpha gene; TUB: partial beta-tubulin gene; SSU: small subunit (18S) of the rRNA gene operon.
Results

Phylogeny
Three phylogenies were generated; the first was based on 56 LSU sequences (including the outgroup *Dothidea sambuci* GenBank aY544681) and was used to determine the familial and ordinal relationships of the studied species (Fig. 1), the second was based on a combined ITS and LSU alignment of 44 isolates (including the outgroup *Saccharata proteae* strain CBS 115206) and was used to determine the genus relationships and species identification within the *Botryosphaeriaceae* (Fig. 2), and the third was based on a combined ITS, LSU, SSU, TEF and TUB alignment of 18 *Darkera* isolates and was used for species identification (Fig. 3).

The first analysis (LSU) (including the outgroup sequence) and the resulting dataset of 773 characters, including alignment gaps which were treated as fifth base, consisted of 604 constant characters, 36 variable parsimony-uninformative characters and 133 parsimony-informative characters. The maximum of 1000 equally most parsimonious trees were retained (TL = 367; CI = 0.638; RI = 0.928; RC = 0.591), the first of which is presented in Fig. 1. The overall topology was identical between the distance tree (data not shown) and the presented parsimony tree (Fig. 1) with some minor rearrangements of terminal clades in the different families. Overall, the parsimony analysis yielded less well-supported nodes compared to the distance analysis. The Dermateaceae was well-supported in both analyses, whereas the Phacidiaceae was only supported in the distance analysis. The *Darkera* clade itself is well-supported in both analyses, although the deeper structure of the sub-clades of the Phacidiaceae collapses into a basal polytomy in the parsimony analysis (see strict consensus branches in Fig. 1). The Phyllostictaceae is well-supported in both analyses, whereas the Botryosphaeriaceae is strongly supported in the distance analysis (98 % bootstrap support) but less so in the parsimony analysis (76 % bootstrap support). The LSU phylogeny based on the current dataset alone does not provide a well-supported topology for the Botryosphaeriaceae and therefore the data was combined with ITS for the second analysis.

The second analysis (combined ITS and LSU alignment) (including the outgroup sequence) and the resulting dataset of 1243 characters, including alignment gaps which were treated as fifth base, consisted of 976 constant characters, 92 variable parsimony-uninformative characters and 175 parsimony-informative characters. Twenty-two equally most parsimonious trees were obtained (TL = 619; CI = 0.577; RI = 0.809; RC = 0.467), the first of which is presented in Fig. 2. In this phylogeny, all genera that are presented by more than one strain or species are supported with a parsimony bootstrap support value of at least 80 %; the only exception is *Diplodia* which is split into two lineages without support for the connecting node. The tiarosporella-like strains are polyphyletic in the tree and therefore novel genera are introduced below to accommodate those not clustering in the *Tiarosporella* clade. Except for *Tiarosporella tritici* (=*Eutiarosporella tritici*, see below) and *T. africana* (=*Eut. africana*, see below), all species in the ITS-LSU phylogeny could be resolved. In the case of this exception, the two species can easily be distinguished based on their TEF or TUB sequences (data not shown).

The third analysis (combined ITS, LSU, SSU, TEF and TUB alignment) was based on the resulting dataset of 2879 characters, including alignment gaps which were treated as fifth base, consisted of 2857 constant characters, 3 variable parsimony-uninformative characters and 19 parsimony-informative characters (TL = 22; CI = 1.0; RI = 1.0; RC = 1.0). Only a single most parsimonious tree was obtained, presented in Fig. 3, which clearly separated the strains belonging to *Darkera picea* from those belonging to *D. parea*.

Taxonomy

Higher order classification:—Leotiomycetes, Phacidiales, Phacidiaceae


Folicicolous. *Ascomata* amphiogenous, scattered to aggregated, black, confluent to elongate-ellipsoid, immersed, subhypodermal, opening by longitudinal rupture, upper layer of dark *textura epidermoidea*; subhyphenum of pale brown pseudoparenchymatal cells, forming a *textura angularis*. *Paraphyses* simple to branched, septate, slightly swollen at apex, smooth, frequently invested in mucilage. *Asci* clavate, 8-spored, apex slightly flattened, staining positive in Meltzer’s reagent. *Ascospores* biseriate, ellipsoid to subreniform, aseptate, guttulate, hyaline, becoming pale brown. *Conidiomata* globose, immersed to erumpent, brown, opening by means of an irregular rupture; wall of 3–6 layers of...
FIGURE 1. The first of 1000 equally most parsimonious trees (TL = 367; CI = 0.638; RI = 0.928; RC = 0.591) resulting from a parsimony analysis of the LSU (28S) sequence alignment. The bootstrap support values are indicated at the nodes (parsimony bootstrap / distance with HKY85 model bootstrap; only values >74%) and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Orders are indicated in darker blue and orange blocks and family names in light blue and light brown blocks. Species names of interest to this study are shown in bold text. The tree was rooted to Dothidea sambuci (GenBank AY544691).
FIGURE 2. The first of 22 equally most parsimonious trees (TL = 619; CI = 0.577; RI = 0.809; RC = 0.467) resulting from a parsimony analysis of the combined ITS and LSU alignment representing genera in the Botryosphaeriaceae. The bootstrap support values are indicated at the nodes and the scale bar represents the number of changes. Thickened branches reflect those branches present in the strict consensus tree. Genus names in light blue and light brown blocks and abbreviated genus names used for the species follow from the genus name used for the corresponding clade. Species names of interest to this study are shown in bold text. The tree was rooted to *Saccharata proteae* (strain CBS 115206; ITS GenBank KF531812, LSU GenBank KF531812).
brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity, hyaline, smooth, ampulliform to subcylindrical, proliferating percurrently at apex, mono- to polyphialidic. *Paraphyses* intermingled among conidiogenous cells, hyaline to pale brown, smooth to verruculose, septate, subcylindrical with obtuse ends. *Conidia* solitary, hyaline, smooth, guttulate, subcylindrical to fusoid-ellipsoid, straight to curved, apex apiculate, tapering at base to truncate hilum; apex with flared mucoid appendage.

**Type species:**—*Darkera parca* H.S. Whitney, J. Reid & Piroz.


**Note:**—This taxon is known to occur on *Abies* spp., with conidia being (29–)36–42 × (7.5–)8–9 µm (Karadžić 1998). A detailed description and illustration is provided by Nag Raj (1993). Because *T. abietis* is not congeneric with the genus *Tiarosporella*, we propose to use the name of the sexual morph, *Darkera*, for *D. abietis* and other taxa congeneric with it (Whitney et al. 1975). The asexual morph of *Darkera* resembles species of *Phacidium* (= *Ceuthospora*, Crous et al. 2014), but the latter tends to have multilocular conidiomata with several semi-papillate ostioles, smaller conidia and branched conidiophores. Species of *Darkera* are endophytic, and possibly weakly pathogenic on conifers (Müller & Hantula, 1998).

**FIGURE 3.** The single most parsimonious circle tree (TL = 22; CI = 1.0; RI = 1.0; RC = 1.0) resulting from an unrooted parsimony analysis of the combined ITS, LSU, SSU, TEF and TUB alignment strains of *Darkera*. Host countries are shown next to the culture accession number. The scale bar represents the number of changes.
Darkera durmitorensis (Karadžić) Crous, comb. nov. MycoBank MB811245


Note:—This taxon is known to occur on Abies spp., with conidia being 33–60 × 9.5–13.5 µm (Karadžić 1998). Based on its morphology (large unilocular conidiomata and long, wide conidia) and ecology (occurring on Picea spp.), it clearly is a species of Darkera, and not Tiarosporella, and hence a new combination is proposed for it.

Darkera parca H.S. Whitney, J. Reid & Piroz., Canadian Journal of Botany 53: 3053 (1975); Fig. 4

Synonyms: Sphaeropsis parca Berk. & Broome, Annals and Magazine of Natural History 5: 420 (1850)
Phoma parca (Berk. & Broome) Sacc., Syllogis fungorum (Abellini) 3: 100 (1884)
Macrophoma parca (Berk. & Broome) Berl. & Voglino, Atti della Società Veneziana-Trentina-Istriana di Scienze Naturali 10: 191 (1886)

Conidiomata globose, immersed to erumpent, brown, up to 250 µm diam, opening by means if an irregular rupture; wall of 3–6 layers of brown textura angularis. Conidiophores reduced to conidiogenous cells. Conidiogenous cells lining the inner cavity, hyaline, smooth, ampulliform to subcylindrical, proliferating percurrently at apex, mono- to polyphialidic, 8–15 × 3–4 µm. Paraphyses intermingled among conidiogenous cells, hyaline to pale brown, smooth to verruculose, 0–4-septate, subcylindrical with obtuse ends, 30–55 × 2–3 µm. Conidia solitary, hyaline, smooth, guttulate, fusoid-ellipsoid to subcylindrical, straight to curved, apex apiculate, tapering at base to truncate hilum, 2 µm diam, (22–)25–30(–41) × (6–)7(–7.5) µm; apex with flared mucoid appendage, up to 15 µm long, 13 µm diam (based on CPC 23904).


Culture characteristics:—Colonies dirty white on all media, with moderate aerial mycelium and feathery margins, covering dish in 1 mo.


Notes:—Although the connection between the sexual and asexual morph was based on association, and not
confirmed via culture studies, we regard this link as probably correct, as tiarosporella-like morphs have been linked to more than one species of Darkera (Whitney et al. 1975). Furthermore, the present fungus corresponds very well with the asexual morph identified by Whitney et al. (1975) from Canada as *T. parca* (conidia (20–)23–40 × 4–6(–7) µm), and linked to *Darkera parca*. However these dimensions differ slightly from those provided later by Nag Raj (1993) for *D. parca*, which are larger, (29–)35–43 × 9–12 µm. It could well be that the original species described from the UK as *Sphaeropsis parca* Berk. & Broome is not conspecific with the Canadian *D. parca*. For this reason we propose to retain the name *D. parca* H.S. Whitney, J. Reid & Piroz. 1975 for the collections from Canada and Siberia. Further cultures and molecular data need to be studied to resolve the issue if *Darkera parca* from Canada is conspecific with *Sphaeropsis parca* Berk. & Broome 1850 from the UK.

**Darkera picea** Crous & M.M. Müller, *sp. nov.* MycoBank MB811246; Fig. 5

**Etymology:**—Named after the host genus from which it was collected, *Picea*.

*Conidiomata* globose, immersed to erumpent, brown, up to 250 µm diam, opening by means of an irregular rupture; wall of 3–6 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity, hyaline, smooth, ampulliform to subcylindrical, proliferating percurrently at apex, mono- to polyphialidic, 5–20 × 4–5 µm. *Paraphyses* intermingled among conidiogenous cells, hyaline to pale brown, smooth to verruculose, 0–2-septate, subcylindrical with obtuse ends, 30–50 × 3–4 µm. *Conidia* solitary, hyaline, smooth, guttulate, subcylindrical to fusoid-ellipsoid, straight to curved, apex apiculate, tapering at base to truncate hilum, 2–4 µm diam, (36–)40–46(–53) × (7–)8–9(–10) µm; apex with flared mucoid appendage, up to 25 µm long, 20 µm diam (based on CPC 23897).


**Culture characteristics:**—Colonies dirty white on all media, with moderate aerial mycelium and feathery margins, covering dish in 1 mo.

*Darkera pseudotsugae* (H.S. Whitney, J. Reid & Piroz.) Crous, *comb. nov.* MycoBank MB811247


Note:—This taxon is known to occur on *Pseudotsuga* spp., with conidia being (33–)40–65 × (4–)6–7 µm (Karadžić 1998). Based on its ecology (on conifer needles), as well as morphology (large unilocular conidiomata and long, wide conidia), it clearly is better accommodated in *Darkera* rather than *Tiarosporella*, and hence a new combination is herewith proposed for this taxon.

Higher order classification:—Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae

*Eutiarosporella* Crous, *gen. nov.* MycoBank MB811248

Etymology:—Named after its morphological similarity to the genus *Tiarosporella*.

Distinguished from *Tiarosporella* by having conidiomata with long necks, and having holoblastic conidiogenesis. Similar to *Marasasiomyces*, except conidiomata frequently in clusters. *Conidiomata* pycnidial, uni- to multilocular, dark brown to black, globose, rostrate with elongated necks, with or without setae, aggregated in clusters. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner cavity, holoblastic, determinate, cylindrical, hyaline, smooth. *Conidia* solitary, hyaline, smooth, thin-walled, straight, ovoid to fusoid, apex obtuse, base truncate, with a cone-like mucoid apical appendage.

Type species:—*Eutiarosporella tritici* (B. Sutton & Marasas) Crous

*Eutiarosporella africana* (Jami, Gryzenh., Slippers & M.J. Wingf.) Crous, *comb. nov.* MycoBank MB811249


Specimen examined:—SOUTH AFRICA. Gauteng Province, Pretoria, from healthy wood section of *Celtis africana*, Nov. 2011, F. Jami & M. Gryzenhout (holotype PREM 60866, culture ex-type CMW 38423 = CBS 133854).

*Eutiarosporella tritici* (B. Sutton & Marasas) Crous, *comb. nov.* MycoBank MB811250


*Eutiarosporella urbis-rosarum* (Jami, Gryzenh., Slippers & M.J. Wingf.) Crous, *comb. nov.* MycoBank MB811251

Specimen examined:—SOUTH AFRICA. Free State Province, Bloemfontein, healthy wood of Vachellia karroo, June 2008, M. Gryzenhout (holotype PREM 60698, culture ex-type CBS 130405).

Marasasiomyces Crous, gen. nov. MycoBank MB811252

Etymology:—Named after Walter Friedrich Otto Marasas, who collected this fungus in the Karoo, South Africa.

Distinguished from Tiarosporella by having conidiomata with long necks, covered in brown setae, and having holoblastic conidiogenesis. Similar to Eutiarosporella, but conidiomata not in clusters.

Conidiomata pycnidial, dark brown to black, rostrate with elongated necks, covered in brown, simple, septate, smooth to verruculose setae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells lining the inner cavity, holoblastic, determinate, cylindrical, hyaline, smooth. Conidia solitary, hyaline, smooth, thin-walled, straight, fusiform, apex obtuse, base truncate, with a cone-like mucoid apical appendage.

Type species:—Marasasiomyces karoo (B. Sutton & Marasas) Crous

Marasasiomyces karoo (B. Sutton & Marasas) Crous, comb. et stat. nov. MycoBank MB811253


Conidiomata pycnidial, dark brown to black, rostrate with elongated necks, covered in brown, simple, septate, smooth to verruculose setae. Conidiophores reduced to conidiogenous cells. Conidiogenous cells lining the inner cavity, holoblastic, determinate, apex, cylindrical, hyaline, smooth, 12–18× 1.5–2.5 µm. Conidia solitary, hyaline, smooth, thin-walled, straight, fusiform, apex obtuse, base truncate, 21–28 × 5–8 µm, with a cone-like mucoid apical appendage.

Specimen examined:—SOUTH AFRICA. Cape Province: Colesberg, on dead stems of Eriocephalus sp., Feb. 1971, W.F.O. Marasas (holotype PREM 44967, isotype IMI 186782, culture ex-type CBS 118718).

Notes:—The peculiar conidiomata with elongated necks, covered in brown setae, was commented on when this fungus was originally described (Sutton & Marasas 1976), and also illustrated subsequently (Crous et al. 2006, fig. 7). Furthermore, in a study elucidating the conidiogenesis of this fungus, Roux et al. (1990) did not find any evidence of percurrent proliferation, while this feature is again prominent in Tiarosporella s.str.

Mucoharknessia Crous, R.M. Sánchez & Bianchin., gen. nov. MycoBank MB811254

Etymology:—Muco, derived from the mucoid appendage, and Harknessia (resembling the genus).

Mucoharknessia resembles Harknessia (Harknessiaceae, Diaporthales), but is distinguished from that genus by having pycnidia that lack furfuraceous tissue surrounding its ostiole, and conidia that have a mucoid apical appendage.

Foliicolous. Conidiomata immersed, separate or aggregated, pycnidial, unilocular, globose to subglobose, blackish on leaves; ostiole subepidermal, circular to subcircular, opening onto the abaxial side of leaves by means of a longitudinal split in epidermis. Peridium arranged in two layers, the external stromatic, with brown cells of textura angularis; the internal conformed by flattened, hyaline cells, 10–15 µm thick. Conidiophores reduced to conidiogenous cells, lining the conidiomatal cavity. Conidiogenous cells lageniform to subcylindrical, smooth, covered in mucus, hyaline; proliferating several times percurrently at apex, with flared collarette visible. Conidia oval to ellipsoidal, appendaged, thick-walled, smooth to finely verruculose, lacking striations, brown; apical appendage extracellular (Type B, sensu Nag Raj 1993), mucilaginous, irregular, smooth, hyaline; basal appendage tubular, thin walled, smooth, hyaline, often collapsing. Microconidia not seen.

Type species:—Mucoharknessia cortaderiae Crous, R.M. Sánchez & Bianchin.

Mucoharknessia cortaderiae Crous, R.M. Sánchez & Bianchin., sp. nov. MycoBank MB811255; Fig. 6

Etymology:—After the genus Cortaderia on which the fungus was first found.
Conidiomata immersed, separate or aggregated, pycnidial, unilocular, globose to subglobose, blackish on leaves, 110–315 µm high, 250–350 µm diam. Ostiole subepidermal, circular to subcircular, opening onto the abaxial side of leaves by means of a longitudinal split in epidermis; lacking furfuraceous tissue that surrounds ostiolar openings in Harknessia s.str. Peridium arranged in two layers, the external stromatic, with brown cells of textura angularis, 35–45 µm thick; the internal conformed by flattened, hyaline cells, 10–15 µm thick. Conidiophores reduced to conidiogenous cells, lining the conidiomatal cavity. Conidiogenous cells lageniform to subcylindrical, smooth, covered in mucus, hyaline, 7–18 µm long, 3–6 µm diam at the base, 2–4 µm diam at the apex; proliferating several times percurrently at apex, with flared collarette visible. Conidia oval to ellipsoidal, appendaged, thick-walled, smooth to finely verruculose, lacking striations, brown, (18–)21–27(–39) × (9–)11–12(–17) µm; apical appendage extracellular (Type B, sensu Nag Raj 1993), mucilaginous, irregular, smooth, hyaline, 3–5 µm long, best seen with India ink; basal appendage tubular, thin walled, smooth, hyaline, 1–5 µm long, 3–5 µm diam, often collapsing. Microconidia not seen.

![Figure 6](image-url)  

**Cultural characteristics:**—Colonies covering the dish in 2 wk, with sparse aerial mycelium, and even feathery margins; surface on MEA and PDA olivaceous grey, reverse iron grey.

**Specimen examined:**—ARGENTINA. Buenos Aires Province, Punta Alta, 38°47′27.6″S 62°6′48.6″W, on leaves of Cortaderia selloana (Schult. & Schult. f.) Asch. & Graebn. (Poaceae), 29 Mar. 2011, F.E. Anderson (holotype BBB, (MVB 1502), isotype CBS h-21853, culture ex-isotype CBS 131032 = CPC 19974, CPC 22208, 22209).

**Additional specimens examined:**—all on leaves of Cortaderia selloana; ARGENTINA. Buenos Aires Province: La Paz, 35°21′32.5″ W59°19′57.2″, 30 May 2011, F.E. Anderson, C10; Miramar, 38°13′12″ W57°42′51″, 18 Jul. 2011, L. Gallego, C14-1; Monte Hermoso, 38°59′9.5″ W61°7′42.9″, 24 Apr. 2011, F.E. Anderson, C7; Tandil, 37°18′17.5″ W59°8′4.9″, 23 Apr. 2011, L. Gallego, C8-1, Tandil, 37°18′17.5″ W59°8′4.9″, 18 Jul. 2011, L. Gallego, C8-3.

**Notes:**—Conidiomata interveinal, associated with elongated, pale brown to yellowish or orange-brown necrotic leaf blade sections, most likely as a secondary invader, which proved to be rather uncommonly encountered. With its unilocular conidiomata, and pigmented, appendaged conidia, it is somewhat reminiscent of Harknessia (Crous et al. 2000).
et al. 2012) and Macrophomina (Sarr et al. 2014). Phylogenetically however, it proved to be allied to genera in the Tiarosporella complex in the Botryosphaeriaceae (Fig. 1), which was quite unexpected.


Foliicolous. Conidiomata pycnidial, aggregated, immersed, depressed, globose, mostly irregularly multilocular in a stroma, dark brown, ostiolate; wall of 3–6 layers of brown textura angularis. Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, lining the inner cavity, subcylindrical to ampulliform; conidiogenesis holoblastic, lacking phialides with percurrent proliferation or periclinal thickening. Conidia subcylindrical to clavate or narrowly ellipsoid, apex obtuse, base truncate, aseptate, smooth, hyaline, granular, with an apical cone-shaped appendage which splits into up to four tentaculiform undulate appendages.

**Type species:**—*Sakireeta madreeya* Subram. & K. Ramakr.

**Sakireeta madreeya** Subram. & K. Ramakr., Journal of the Indian Botanical Society 36: 84 (1957); Fig. 7


Foliicolous. Conidiomata pycnidial, aggregated, immersed, depressed, globose, mostly irregularly multilocular in a stroma, dark brown, ostiolate; wall of 3–6 layers of brown textura angularis. Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline, smooth, lining the inner cavity, subcylindrical to ampulliform, 4–7 × 3–5 µm; conidiogenesis holoblastic, lacking phialides with percurrent proliferation or periclinal thickening. Conidia (15–)18–25(–30) × (4–)5–6(–7) µm, subcylindrical to clavate or narrowly ellipsoid, apex obtuse, base truncate, aseptate, smooth, hyaline, granular, with an apical cone-shaped appendage which splits into up to four tentaculiform undulate appendages.

![FIGURE 7. Sakireeta madreeya (CBS 532.76). A. Conidiomata on PNa. B–D. Conidiogenous cells. E, F. Conidia. Scale bars: A = 250 µm, all others = 10 µm.](image)

**Culture characteristics:**— Colonies spreading, flat, with moderate, cottony aerial mycelium, and feathery margins. On MEA surface dirty white, reverse olivaceous-black. On OA surface olivaceous-grey.

**Specimens examined:**— INDIA. Madras, Choolai, on dead culm of *Aristida setacea*, 27 Sept. 1951, K. Ramakrishnan (holotype MUBL 631); Kurukshetra Univ., undetermined grass host, July 1976, R.S. Mehrotra, CBS H-21854, culture CBS 532.76.

**Notes:**—The culture originally deposited as *Tiarosporella madreeya* from India (CBS 532.76) closely corresponds with the morphology of the type specimen, and therefore we regard it as authentic. However, as the host was never stipulated, and the laboratory records of Prof. R.S. Mehrotra (communicated via Dr K.C. Rajeshkumar) indicate that it was collected as a saprobe from grasses buried in soil for decomposition. As it is impossible to accurately identify the host, we thus refrain from designating it as epitype for the genus.

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Of interest is that the type of Tiarosporella, *T. paludosa*, has solitary unilocular conidiomata, whereas those of *Sakireeta madreya* are aggregated in a stroma, and plurilocular. Furthermore, *T. paludosa* has percurrently proliferating conidiogenous cells, whereas those of *Sakireeta madreya* are holoblastic. Once more species of these two genera have been collected and subjected to DNA analysis to confirm their generic placement, it will be possible to confirm if these characters are also valuable at the generic level in distinguishing Tiarosporella from Sakireeta.

**Tiarosporella** Höhn., Berichte der Deutschen Botanischen Gesellschaft 37: 159 (1919)

Foliicolous, rarely caulicolous. **Conidiomata** pycnidial, separate, immersed, globose to depressed, unilocular, dark brown, with central substomatal ostiole; wall of brown textura angularis. **Conidiophores** reduced to conidiogenous cells lining the inner cavity. **Conidiogenous cells** hyaline, smooth, subcylindrical to ampulliform, encased in mucus, proliferating percurrently near the apex. **Conidia** hyaline, smooth, solitary, subcylindrical to subclavate, apex subobtuse, base truncate, rarely with marginal frill, aseptate, bearing 2–4 tentaculiform, undulate apical mucoid appendages.

The conidium is initially covered in a mucoid sheath, which splits longitudinally, resulting in apical tentaculiform appendages.

**Type species:**—*Tiarosporella paludosa* (Sacc. & Fiori ex P. Syd.) Höhn.

**Tiarosporella paludosa** (Sacc. & Fiori) Höhn., Berichte der Deutschen Botanischen Gesellschaft 37: 159 (1919); Fig. 8

Basionym: *Neottiospora paludosa* Sacc. & Fiori, Hedwigia Beiblätter 38: 137 (1899)


**Culture characteristics:**—Colonies fast growing, covering the dish in 2 wk; grey olivaceous on surface and olivaceous black in reverse, with fluffy aerial mycelium and even, feathery margins.


**Notes:**—*Tiarosporella paludosa* occurs rather commonly in Germany on *Carex* spp., *Eriophorum polystachium* and *Trichophorum cespitosum* (= *Scirpus caespitosus*) (Sutton 1980, Nag Raj 1993), and is obviously widely distributed in Europe. It is also known to occur in Canada and the USA (Nag Raj 1993). The present collection closely matches the morphology of the holotype, and is also from Germany, where this taxon occurs commonly on *Carex, Eriophorum* and *Trichophorum* (Sutton 1980, Nag Raj 1993). Phylogenetically it is identical to another culture of *T. paludosa* from *Eleocharis palustris* (CBS 114650; sterile) collected in Sweden (Table 1), justifying CBS H-21855 as an excellent epitype specimen for the taxon, which also fixes the genetic application of the name.

**Discussion**

Results from the present study revealed that the genus *Tiarosporella s.lat.* is actually poly- and paraphyletic. *Tiarosporella*-like taxa cluster in the Phacidiaeae, and Botryosphaeriaceae. Species of *Tiarosporella s.str.* belong to the Botryosphaeriaceae. Those species clustering in the Phacidiaeae (see Crous *et al.*, 2014), are associated with needle diseases of conifers (Karadžić 1998, Müller & Hantula 1998), and would be better allocated to the genus *Darkera*, for which a new species, *D. picea*, occurring on *Picea* spp. in Finland, Norway and Switzerland is introduced. This species is closely related to *D. parca* which occurs according to morphological characteristics both in Siberia and Canada and possibly also in Europe. Further collections are required, however, to resolve the status of *D. parca* in northern boreal forests, to determine if this is a morphologically variable taxon, or if several different species are involved, the species in the UK having somewhat larger conidia than the species occurring in Canada and Siberia.

Furthermore, the epitypification of *Tiarosporella*, based on *T. paludosa*, allowed us to separate this genus from its close allies in the Botryosphaeriaceae that actually form a subclade (Fig. 2), representing several genera with conidial appendages. This subclade includes genera such as *Botryobambusa* (see Liu *et al.* 2012 fig. 11, though appendage overlooked by the authors), and two new genera, namely *Marasasiomyces*, and *Eutiarosporella*. *Eutiarosporella* is morphologically similar to *Marasasiomyces* (long necked, hairy conidiomata, and holoblastic conidiogenesis), except that it forms conidiomata in clusters, which is not the case in *Marasasiomyces*. The latter two genera are distinguished from *Tiarosporella* by having conidiomata with elongated necks, and holoblastic conidiogenesis, while *Tiarosporella* has globose to depressed, unilocular conidiomata and conidigenous cells with percurrent proliferation. *Marasasiomyces* and *Eutiarosporella* cluster sister to the genus *Mucoharknessia*, which appears harknessia-like in general morphology. The genus *Harknessia* (Harknessiaceae, Diaporthales; Crous *et al.*, 2012) is similar to *Apoharknessia* (conidia with apical apiculus, short basal appendage, and percurrent proliferating conidigenous cells; Lee *et al.* 2004) and *Dwiroopa* (conidia with prominent longitudinal conidial germ slits; Farr & Rossman 2003). *Mucoharknessia* is distinguished from these genera by lacking the brown, furfuraceous margins around the ostioles of conidiomata, and by being allied to the Botryosphaeriaceae. Finally, the genus *Sakireeta* is resurrected, and shown to cluster apart from *Tiarosporella*, having multilocular conidiomata embedded in a brown stroma, which is distinct from the solitary conidiomata of *Tiarosporella s.str.*

In spite of recent studies that have provided molecular support for 18 genera in the Botryosphaeriaceae (Crous *et al.*, 2013, Phillips *et al.*, 2013, Wijayawardene *et al.* 2014), the present study adds four new genera to the family, namely *Eutiarosporella, Marasasiomyces, Mucoharknessia* and *Sakireeta*. Further studies will undoubtedly discover even more genera and species in this family, which appears to have members that are ecologically diverse, inhabiting grasses as well as woody hosts, with life styles including endophytes, saprobes, plant and human pathogens (Phillips *et al.*, 2013, Slippers *et al.*, 2013).
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