Resolving *Tiarosporella* spp. allied to Botryosphaeriaceae and Phacidiaceae

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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 RNA, 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, Phacidiales, systematics.

Running head: *TIAROSPORELLA* AND ALLIED GENERA

Introduction

Several coelomycetous genera with appendaged, hyaline conidia are members of the Botryosphaeriales, namely *Phyllosticta* (Phyllostictaceae; 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 *Botryosphaeriales*, 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 *Tiarospora*, 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 (Phacidiales, Leotiomycetes) (Whitney *et al.* 1975, DiCosmo *et al*. 1984). Species of *Phacidium s.str.* (Phacidiaceae) 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 Phacidiaceae 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 fist 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 EF1-728F (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).

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,

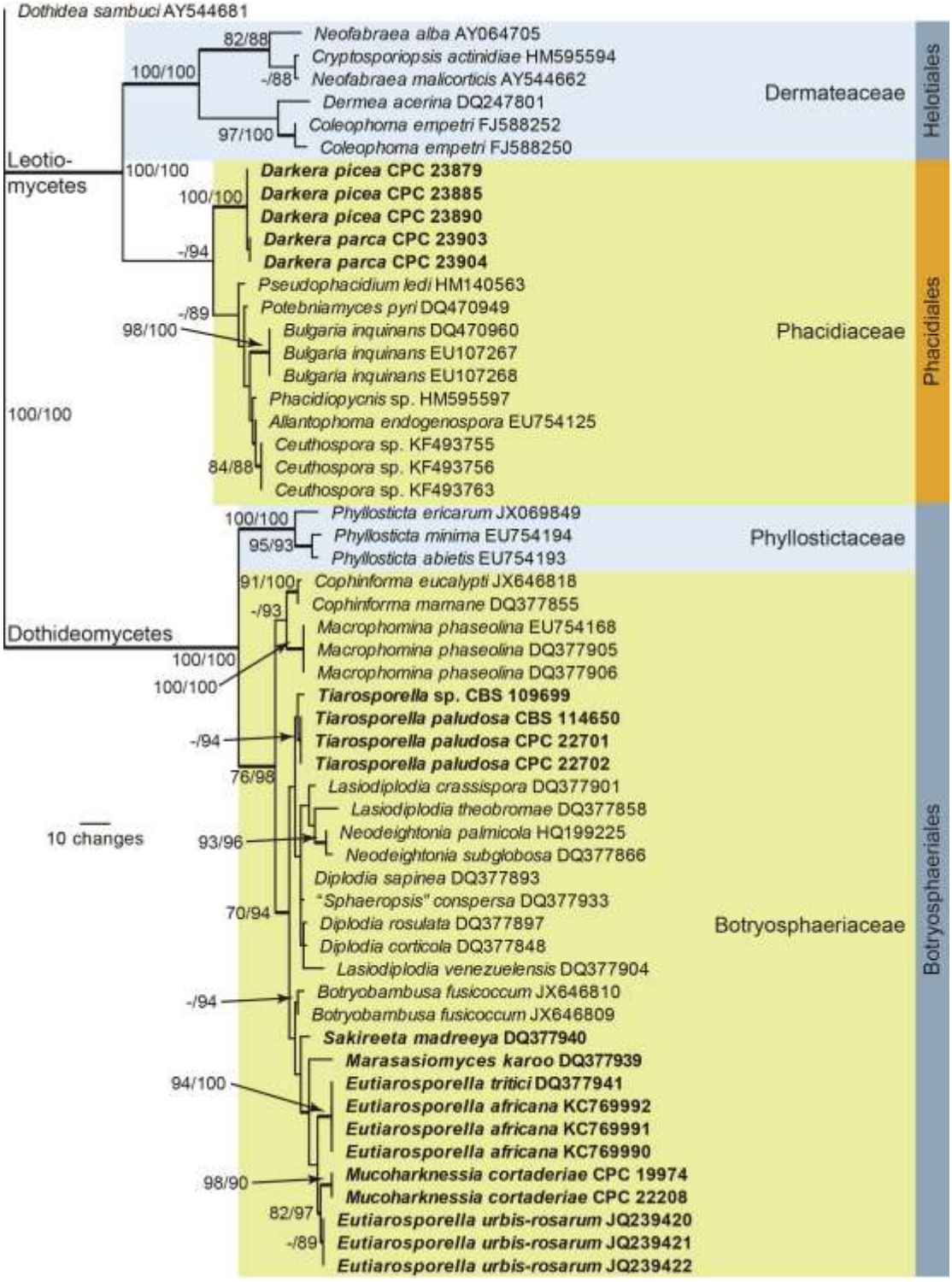

FIGURE 1. The first of 1000 equally most parsimonious trees (TL = 367; CI = 0.638; RI = 0.928; RC = 0.591) resulting from a parsimony analysis of the LSU (28S) sequence alignment. The bootstrap support values are indicated at the nodes (parsimony bootstrap / distance with HKY85 model bootstrap; only 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).

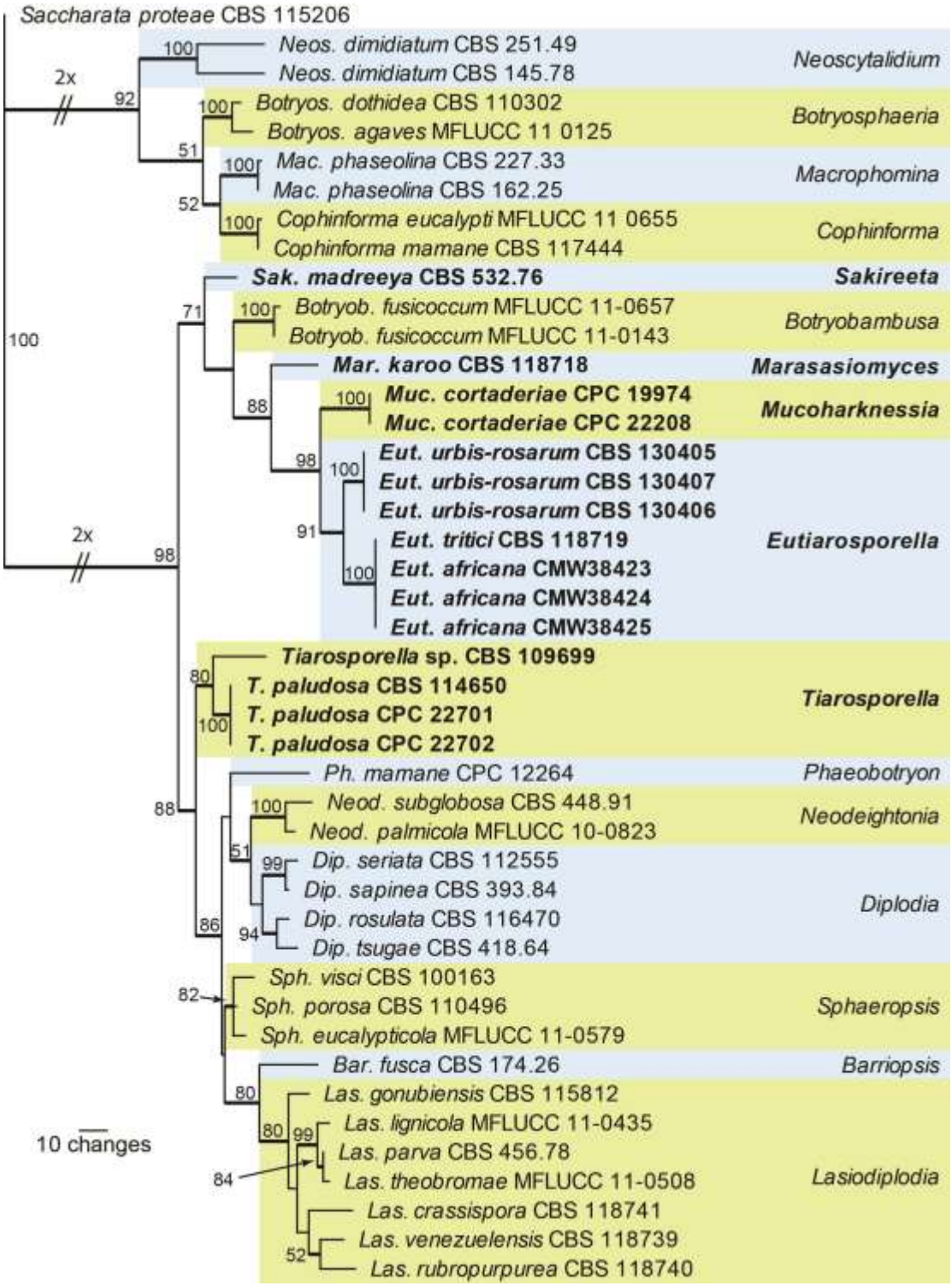

FIGURE 2. The first of 22 equally most parsimonious trees (TL = 619; CI = 0.577; RI = 0.809; RC = 0.467) resulting from a parsimony analysis of the combined ITS and LSU alignment representing genera in the Botryosphaeriaceae. The bootstrap support values are indicated at the nodes and the scale bar represents 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).

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. parca*.

Taxonomy

Higher order classification:— Leotiomycetes, Phacidiales, Phacidiaceae

Darkera H.S. Whitney, J. Reid & Piroz., Canadian Journal of Botany 53: 3052 (1975)

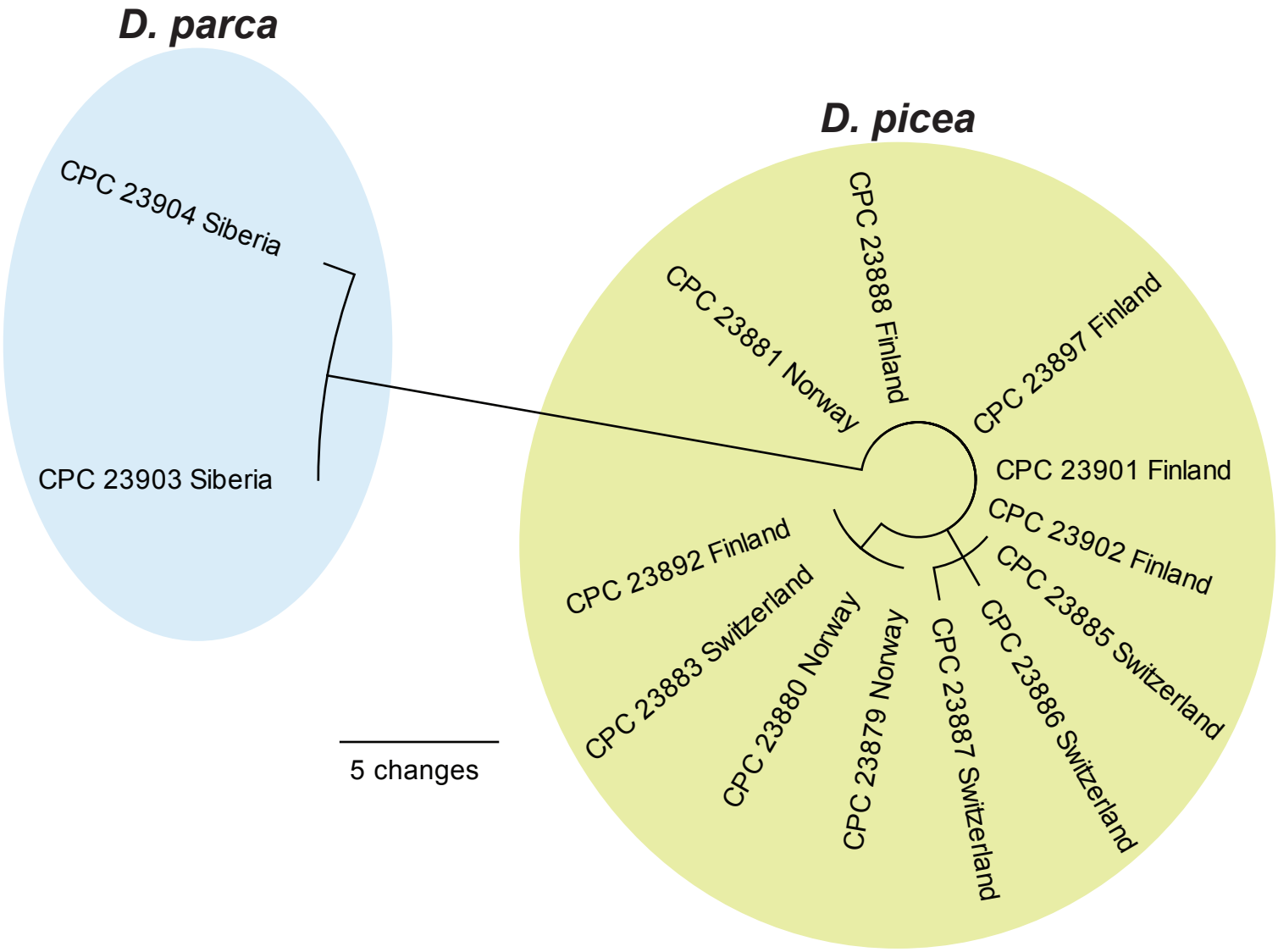

FIGURE 3. The single most parsimonious circle tree (TL = 22; CI = 1.0; RI = 1.0; RC = 1.0) resulting from an unrooted parsimony analysis of the combined ITS, LSU, SSU, TEF and TUB alignment strains of Darkera. Host countries are shown next to the culture accession number. The scale bar represents the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

Foliicolous. *Ascomata* amphigenous, scattered to aggregated, black, confluent to elongate-ellipsoid, immersed, subhypodermal, opening by longitudinal rupture, upper layer of dark *textura epidermoidea*; subhymenium 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 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.

Darkera abietis H.S. Whitney, J. Reid & Piroz., Canadian Journal of Botany 53: 3052 (1975)

Synonym: *Tiarosporella abietis* H.S. Whitney, J. Reid & Piroz., Canadian Journal of Botany 53: 3055 (1975)

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).

Darkera durmitorensis (Karadžić) Crous, **comb. nov.** MycoBank MB811245

Basionym: *Tiarosporella durmitorensis* Karadžić, European Journal of Forest Pathology 28: 148 (1998)

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.), its clearly 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

FIGURE 4. *Darkera parca* (CPC 23904). A. Conidiomata on PNA. B. Conidiomata on OA. C–E. Conidiogenous cells. F–H. Conidia. Scale bars: A = 250 µm, all others = 10 µm.

Synonyms: *Sphaeropsis parca* Berk. & Broome, Annals and Magazine of Natural History 5: 420 (1850)
Phoma parca (Berk. & Broome) Sacc., Sylloge fungorum (Abellini) 3: 100 (1884)
Macrophoma parca (Berk. & Broome) Berl. & Voglino, Atti della Società Veneziana-Trentina-Istriana di Scienze Naturali 10: 191 (1886)
Sirococcus parcus (Berk. & Broome) M. Morelet, as *"parca"*, Bulletin de la Société des Sciences Naturelles et d'Archéologie de Toulon et du Var 205: 9 (1973)
Tiarosporella parca (Berk. & Broome) H.S. Whitney, J. Reid & Piroz., Canadian Journal of Botany 53: 3055 (1975)

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.
Specimens examined:— SIBERIA. Buriatia, Zum Murino, Tunkinski-valley, on needles of *P. abies*, 14 Nov. 2008, *M. Müller*, Ir 406 = CPC 23903; roadside between Zum Murino and Irkutsk, healthy needles of needles of *P. abies* var. *obovata*, 14 Nov. 2008, *M. Müller*, Ir 419 = CPC 23904.
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

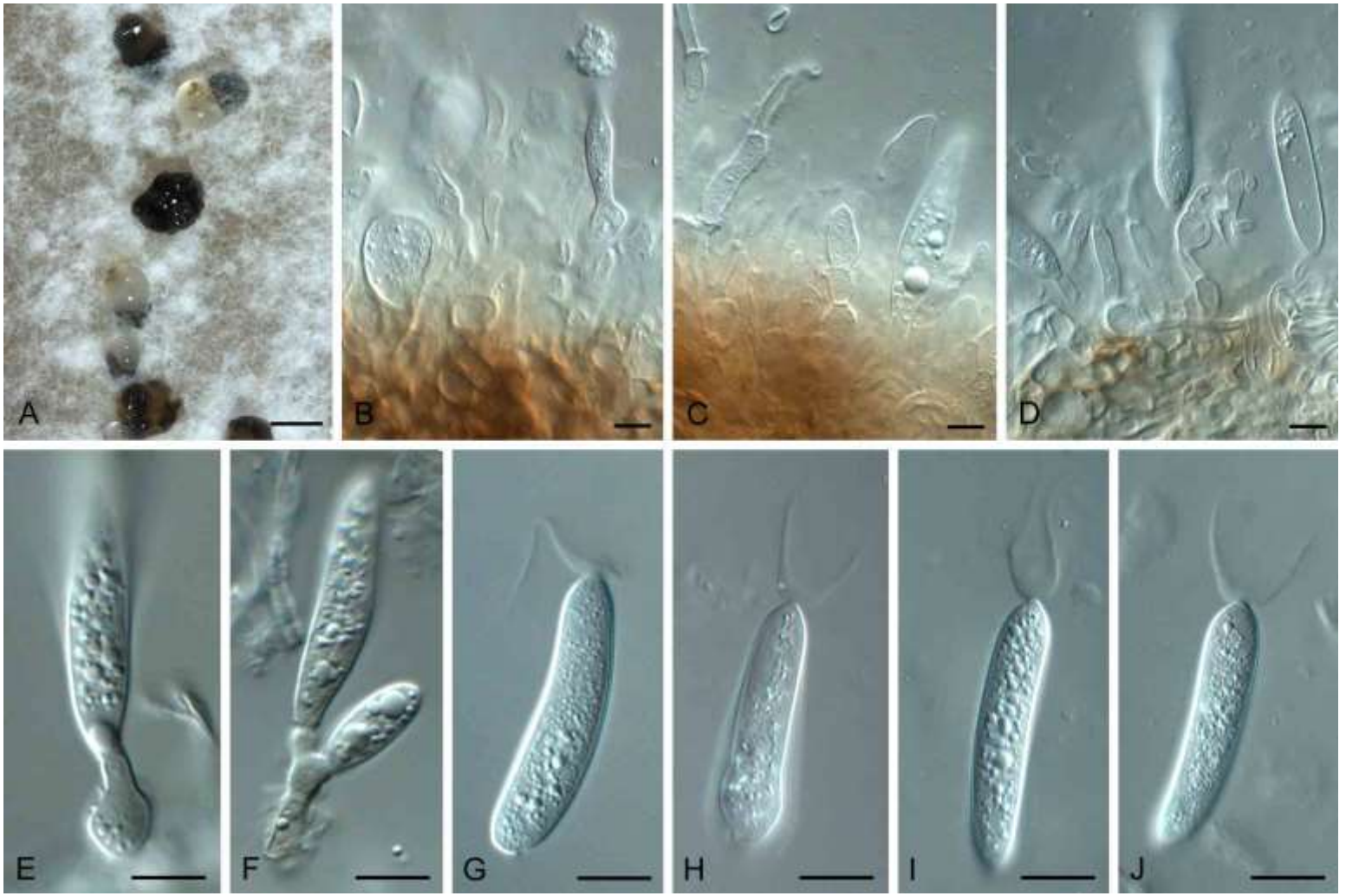

FIGURE 5. *Darkera picea* (CPC 23879). **A.** Conidiomata on OA. **B–F.** Conidiogenous cells. **G–J.** Conidia. Scale bars: A = 250 µm, all others = 10 µm.

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.
Specimens examined:— FINLAND. Tuusula, Ruotsinkylä, on needles of *Picea abies*, 10 Sep. 1995, *A.-M. Hallaksela*, Tp D1 = CPC 23888; Tp T3 = 23890, Tp Q1 = 23892; 1994, *H. Solheim*, 89-2089-18 = CPC 23895; 1994, *H. Solheim*, (holotype CBS H-21852, culture ex-type 89-2090-16 = CPC 23897 = CBS 138576); Apr. 2008, *M.M. Müller*, He 392 = CPC 23900, He 394 = CPC 23901, He 397 = 23902. NORWAY. Mellesmo, Pasvik, on needles of *P. abies*, 1994, H. Solheim, 87-1491-1 = CPC 23879, 87-1491-2 = CPC 23880; Langtjern, 91-727-28 = CPC 23881; Nedstrand, 92-625-69 = CPC 23882. Switzerland, Lägern, on needles of *P. abies*, 30 Sep. 1986, *T. Sieber*, 90.140 = CPC 23883; Fiesch, 90.148 = CPC 23884; Lägern, 90.154 = CPC 23885l; Davos, 90.155 = CPC 23886; 90.157 = CPC 23887.

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

Basionym: *Tiarosporella pseudotsugae* H.S. Whitney, J. Reid & Piroz., Canadian Journal of Botany 53: 3057 (1975)

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

Basionym: *Tiarosporella africana* Jami, Gryzenh., Slippers & M.J. Wingf., Fungal Biology 118: 174 (2014)

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

Basionym: *Tiarosporella tritici* B. Sutton & Marasas, Transactions of the British Mycological Society 67: 74 (1976)

Specimen examined:— SOUTH AFRICA. Free State Province: Heilbron, on *Triticum aestivum*, 18 Jan. 1973, *W.F.O. Marasas* (holotype PREM 44966, isotype IMI 186786, culture ex-type CBS 118719).

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

Basionym: *Tiarosporella urbis-rosarum* Jami, Gryzenh., Slippers & M.J. Wingf., Cryptogamie, Mycologie 33: 256 (2012)

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

Basionym: *Tiarosporella graminis* var. *karoo* B. Sutton & Marasas, Transactions of the British Mycological Society 67: 73 (1976)

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, 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.

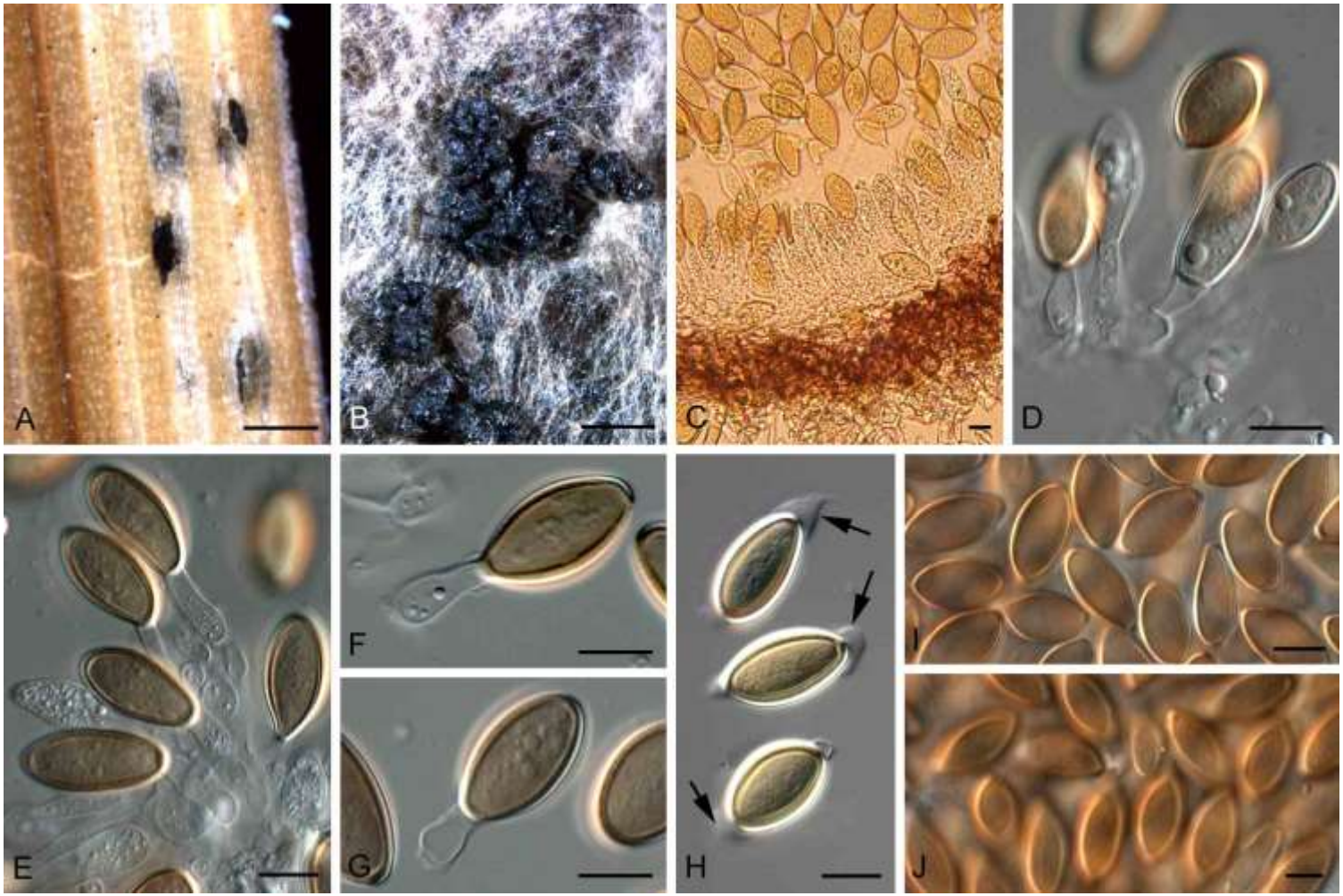

FIGURE 6. *Mucoharknessia cortaderiae* (CPC 19974). **A.** Conidiomata on leaf blade of *Cortaderia selloana*. **B.** Aggregated conidiomata forming on PDA. **C.** Vertical section through a pycnidium, showing wall anatomy. **D–G.** Conidiogenous cells giving rise to conidia (note collarettes, F, G). **H.** Appendaged conidia with arrows indicating apical mucoid caps. **I, J.** Brown, finely verruculose, ellipsoid conidia. Scale bars: A, B = 300 µm, all others = 10 µm.

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.
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, S35°21’32.5” W59°19’57.2”, 30 May 2011, *F.E. Anderson*, C10; Miramar, S38°13’12” W57°42’51”, 18 Jul. 2011, *L. Gallego*, C14-1; Monte Hermoso, S38°59’9.5” W61°7’42.9, 24 Apr. 2011, *F.E. Anderson*, C7; Tandil, S37°18’17.5” W59°8’4.9”, 23 Apr. 2011, *L. Gallego*, C8-1, Tandil, S37°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*. 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.

Sakireeta Subram. & K. Ramakr., Journal of the Indian Botanical Society 36: 83 (1957)

Foliicolous. *Conidiomata* pycnidial, aggregated, immersed, depressed, globose, mostly irregularly multilocular in a stroma, dark brown, ostiolate; wall of 3–6 layers

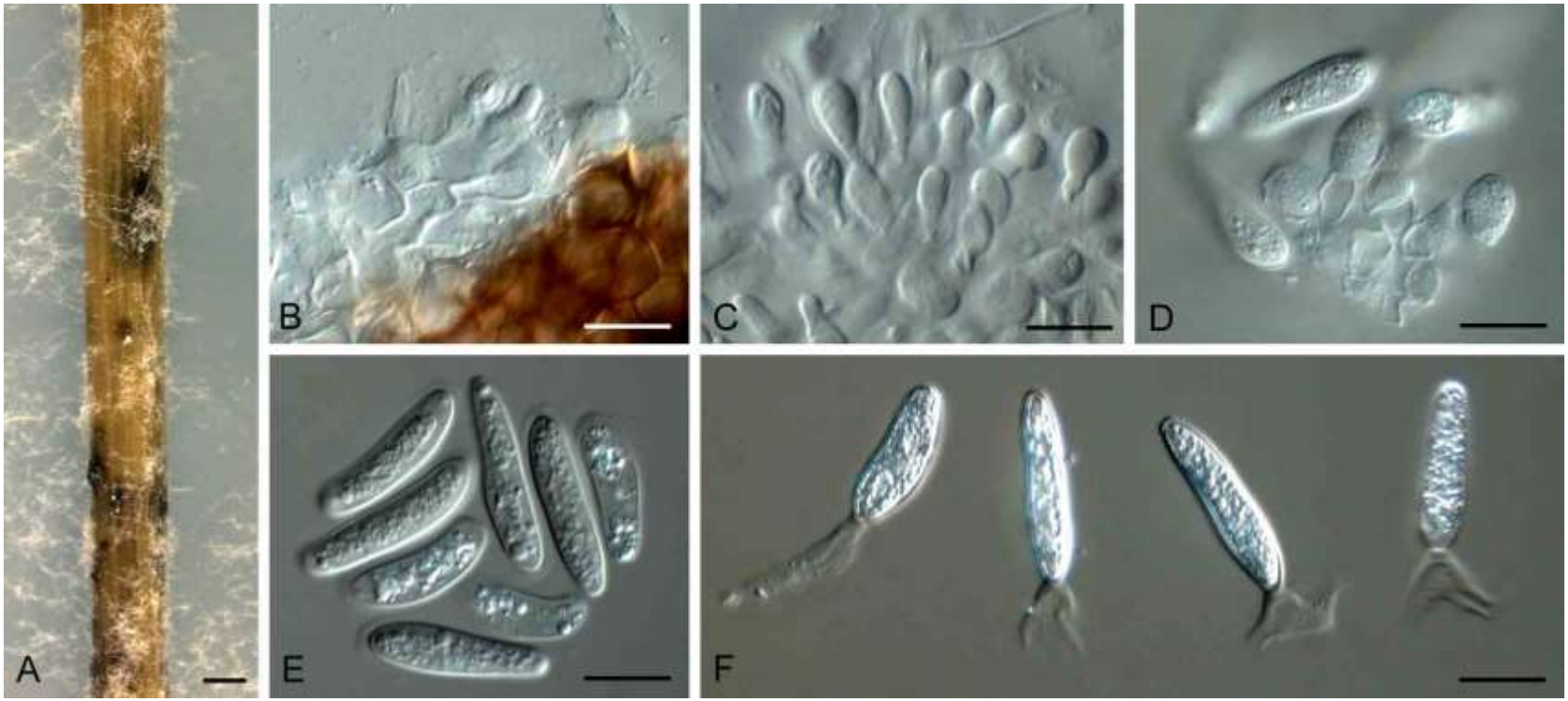

FIGURE 7. *Sakireeta madreeya* (CBS 532.76). **A.** Conidiomata on PNA. **B–D.** Conidiogenous cells. **E, F.** Conidia. Scale bars: A = 250 µm, all others = 10 µm.

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 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

Synonym: *Tiarosporella madreeya* (Subram. & K. Ramakr.) Nag Raj, Canadian Journal of Botany 51: 2470 (1974) [1973]

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.
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.

Of interest is that the type of *Tiarosporella, T. paludosa*, has solitary unilocular conidiomata, whereas those of *Sakireeta madreeya* are aggregated in a stroma, and plurilocular. Furthermore, *T. paludosa* has percurrently proliferating conidiogenous cells, whereas those of *Sakireeta madreeya* 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

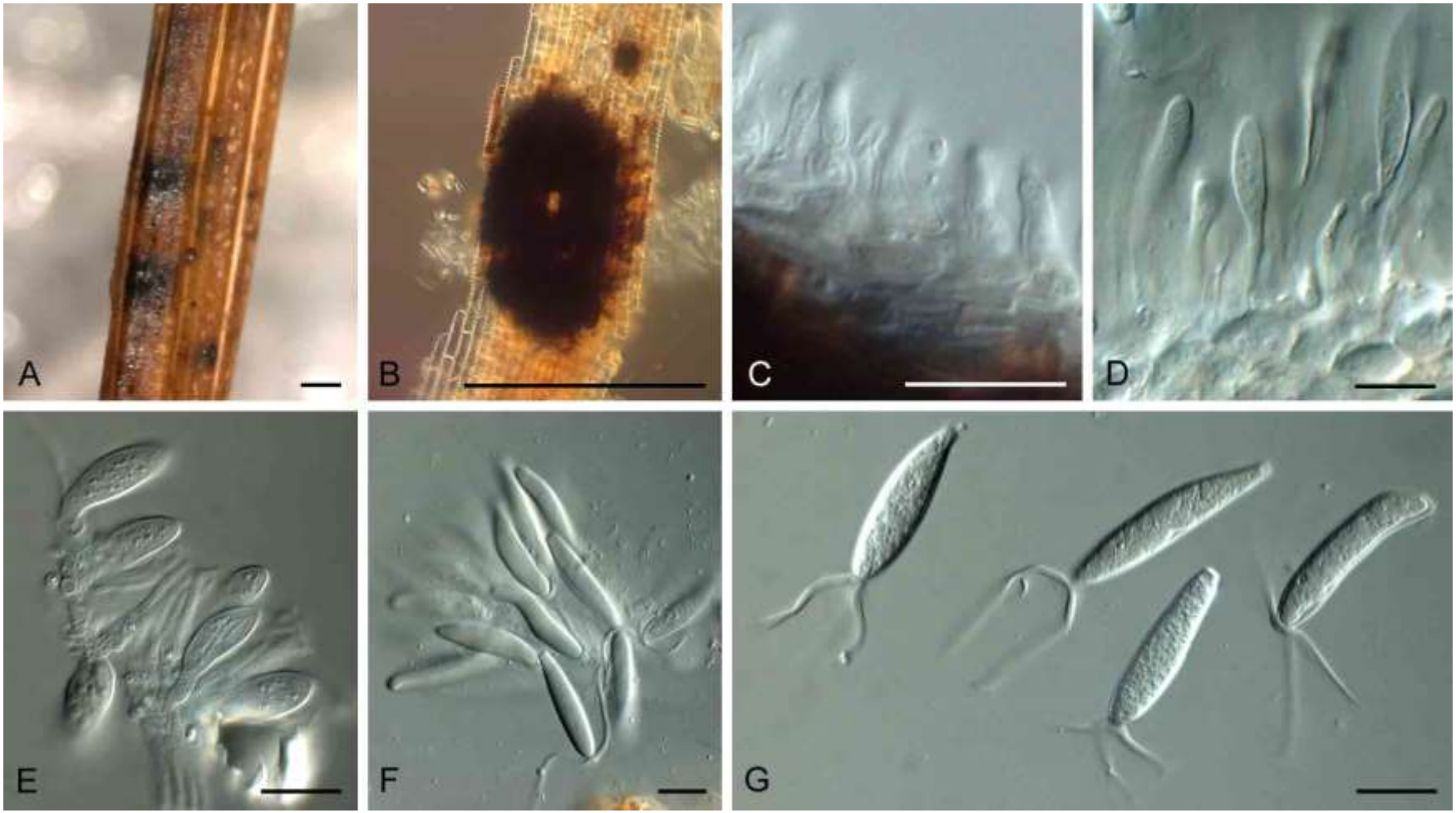

FIGURE 8. *Tiarosporella paludosa* (CPC 22701). **A, B.** Conidiomata on PNA. **C–E.** Conidiogenous cells. **F, G.** Conidia. Scale bars: A, B = 250 µm, all others = 10 µm.

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)
Additional synonyms listed by Nag Raj (1993).

Foliicolous. *Conidiomata* pycnidial, separate, immersed, globose to depressed, 200–350 µm diam, unilocular, dark brown, with central substomatal ostiole; wall of brown *textura angularis*, 20–40 µm thick. *Conidiophores* reduced to conidiogenous cells lining the inner cavity. *Conidiogenous cells* hyaline, smooth, subcylindrical to ampulliform, encased in mucus, 6–15 × 1.5–3.5 µm, proliferating percurrently near the apex. *Conidia* hyaline, smooth, solitary, subcylindrical to subclavate, apex subobtuse, base truncate, 2–3 µm diam, rarely with marginal frill, 1 µm long; conidia aseptate, widest in upper third of conidium, (22–)30–38(–45) × (4–)5–6(–7) µm, 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.
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.
Specimens examined:— GERMANY. Berlin, Zahlendorf, on leaves of *Eriophorum polystachium*, Oct. 1895, *P. Sydow* (holotype in FH, isotype DAOM 130546). NETHERLANDS. Winterswijk in the Korenburgerveen, Latitude 51.990133, Longitude 6.664013, on *Trichophorum cespitosum* subsp. *germanicum*, 28 Apr. 2013, *W. Quaedvlieg* (epitype designated here CBS H-21855 MBT200481, culture ex-epitype CPC 22701, 22702 = CBS 138577). SWEDEN. Åland, Eckerö par., on *Eleocharis palustris*, 14 Sep. 1990, K. & L. Holm, UPSC 3256 = CBS 114650.
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.

TABLE 1. Collection details and GenBank accession numbers of isolates included in this study.

Species	Culture collection no[1]	Substrate	Location	Collector	GenBank Accession no[2]				
					ITS	LSU	TEF	TUB	SSU
Barriopsis fusca	CBS 174.26 ex-type	Twigs of *Citrus* sp.	Cuba	N.E. Stevens	EU673330	DQ377857	–	–	–
Botryobambusa fusicoccum	CBS 134113; CPC 21558; MFLUCC 11-0143 ex-type	Dead culms of *Bambusa* species	Thailand: Lampang Province	R. Phookamsak	JX646792	JX646809	–	–	–
	MFLUCC 11-0657	Dead culms of *Bambusa* species	Thailand: Lampang Province	R. Phookamsak	JX646793	JX646810	–	–	–
Botryosphaeria agaves	CBS 133992; CPC 21559; MFLUCC 11-0125 ex-neotype	Leaves of *Agave* sp.	Thailand: Chiang Rai Province	R. Phookamsak	JX646791	JX646808	–	–	–
Botryosphaeria corticis	CBS 119047; CAP 197 ex-epitype	Stems of *Vaccinium corymbosum*	USA: New Jersey	P.V. Oudemans	DQ299245	EU673244	–	–	–
Botryosphaeria dothidea	CBS 110302; CAP 007	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259092	DQ377851	–	–	–
Cophinforma eucalypti	MFLUCC 11-0655	Dead branch of *Eucalyptus* sp.	Thailand: Chiang Rai Province	M. Doilom	JX646801	JX646818	–	–	–
Cophinforma mamane	CBS 117444; CMW 13416	*Eucalyptus urophylla*	Venezuela	S. Mohali	KF531822	DQ377855	–	–	–
Darkera parca	CPC 23903	Green healthy needle of *Picea abies* var. *obovata*	Siberia	M.M. Müller	KM108354	KM108381	KM108407	KM108452	KM108430
	CPC 23904	Green healthy needle of *Picea abies* var. *obovata*	Siberia	M.M. Müller	KM108355	KM108382	KM108408	KM108453	KM108431
Darkera picea	CPC 23879	Needle of *Picea abies*, pycnidium	Norway	H. Solheim	KM108356	KM108383	KM108409	KM108454	KM108432
	CPC 23880	Needle of *Picea abies*, pycnidium	Norway	H. Solheim	KM108357	KM108384	KM108410	KM108455	KM108433
	CPC 23881	Needle of *Picea abies*	Norway	H. Solheim	KM108358	KM108385	KM108411	KM108456	KM108434
	CPC 23882	Needle of *Picea abies*	Norway	H. Solheim	KM108359	KM108386	KM108412	KM108457	KM108435
	CPC 23883	Needle of *Picea abies*	Switzerland	T. Sieber	KM108360	KM108387	KM108413	KM108458	KM108436
	CPC 23884	Needle of *Picea abies*	Switzerland	T. Sieber	KM108361	KM108388	KM108414	–	KM108437
	CPC 23885	Needle of *Picea abies*	Switzerland	T. Sieber	KM108362	KM108389	KM108415	KM108459	KM108438
	CPC 23886	Needle of *Picea abies*	Switzerland	T. Sieber	KM108363	KM108390	KM108416	KM108460	KM108439
	CPC 23887	–	Switzerland	T. Sieber	KM108364	KM108391	KM108417	KM108461	KM108440
	CPC 23888	Needle of *Picea abies*,	Finland	A.-M. Hallaksela	KM108365	KM108392	KM108418	KM108462	KM108441

		pycnidium							
	CPC 23890	Needle of *Picea abies*, pycnidium	Finland	A.-M. Hallaksela	KM108366	KM108393	KM108419	KM108463	KM108442
	CPC 23892	Needle of *Picea abies*, pycnidium	Finland	A.-M. Hallaksela	KM108367	KM108394	KM108420	KM108464	KM108443
	CPC 23895	*Picea abies*	Finland	H. Solheim	KM108368	KM108395	KM108421	KM108465	KM108444
	CPC 23896	*Picea abies*	Finland	H. Solheim	KM108369	KM108396	KM108422	–	KM108445
	CPC 23897	*Picea abies*	Finland	H. Solheim	KM108370	KM108397	KM108423	KM108466	KM108446
	CPC 23900	Green healthy needle of *Picea abies*	Finland	M.M. Müller	KM108371	KM108398	KM108424	KM108467	KM108447
	CPC 23901	Green healthy needle of *Picea abies*	Finland	M.M. Müller	KM108372	KM108399	KM108425	KM108468	KM108448
	CPC 23902	Green healthy needle of *Picea abies*	Finland	M.M. Müller	KM108373	KM108400	KM108426	KM108469	KM108449
Diplodia mutila	CBS 230.30	*Phoenix dactylifera*	USA: California	–	DQ458886	EU673265	–	–	–
Diplodia rosulata	CBS 116470 ex-type	Seeds of *Prunus africana*	Ethiopia	A. Gure	EU430265	DQ377896	–	–	–
Diplodia sapinea	CBS 393.84 ex-epitype	Cones of *Pinus nigra*	Netherlands: Gelderland	H.A. van der Aa	DQ458895	EU754157	–	–	–
Diplodia seriata	CBS 112555 ex-epitype	Dead stems of *Vitis vinifera*	Portugal	A.J.L. Phillips	AY259094	KF766327	–	–	–
Diplodia tsugae	CBS 418.64 ex-isotype	Branches of *Tsuga heterophylla*	Canada: British Columbia	A. Funk	DQ458888	DQ377867	–	–	–
Eutiarosporella africana	CBS 133854; CMW 38423 ex-type	Healthy wood section of *Celtis africana*	South Africa: Gauteng Province	F. Jami & M. Gryzenhout	KC769956	KC769990	KC769852	KC769903	–
	CBS 135850; CMW 38424	Healthy branches of *Celtis africana*	South Africa: Gauteng Province	F. Jami & M. Gryzenhout	KC769957	KC769991	KC769853	KC769904	–
	CBS 135851; CMW 38425	Healthy branches of *Celtis africana*	South Africa: Gauteng Province	F. Jami & M. Gryzenhout	KC769958	KC769992	KC769854	KC769905	–
Eutiarosporella tritici	CBS 118719; IMI 186786 ex-type	*Triticum aestivum*	South Africa: Free State Province	W.F.O. Marasas	KF531830	DQ377941	KF531809	KF531810	KF531829
Eutiarosporella urbis-rosarum	CBS 130405; CMW 36477 ex-type	Healthy branches of *Acacia karroo*	South Africa: Free State Province	M. Gryzenhout	JQ239407	JQ239420	JQ239394	JQ239381	–
	CBS 130406; CMW 36478 ex-paratype	Healthy branches of *Acacia karroo*	South Africa: Free State Province	M. Gryzenhout	JQ239408	JQ239421	JQ239395	JQ239382	–
	CBS 130407; CMW 36479	Healthy branches of *Acacia karroo*	South Africa: Free State Province	M. Gryzenhout	JQ239409	JQ239422	JQ239396	JQ239383	–
Lasiodiplodia crassispora	CBS 118741; CMW 14691; WAC 12533 ex-type	*Santalum album*	Australia: Western Australia	T.I. Burgess	DQ103550	DQ377901	–	–	–

Lasiodiplodia gonubiensis	CBS 115812; CMW 14077 ex-type	*Syzygium cordatum*	South Africa: Eastern Cape Province	D. Pavlic	DQ458892	DQ377902	–	–	–
Lasiodiplodia lignicola	MFLUCC 11-0435 ex-type	Dead wood	Thailand: Chiang Rai Province	A.D Ariyawansa	JX646797	JX646814	–	–	–
Lasiodiplodia parva	CBS 456.78 ex-type	Cassava field soil	Colombia	O. Rangel	KF766192	KF766362	–	–	–
Lasiodiplodia pseudotheobromae	CBS 447.62	Fruit of *Citrus aurantium*	Suriname	–	EF622081	EU673255	–	–	–
Lasiodiplodia rubropurpurea	CBS 118740; CMW 14700; WAC 12535 ex-type	Canker on *Eucalyptus grandis*	Australia: Queensland	T.I. Burgess	DQ103553	DQ377903	–	–	–
Lasiodiplodia theobromae	MFLUCC 11-0508	Dead twig of *Eucalyptus* sp.	Thailand: Chiang Rai Province	M. Doilom	JX646799	JX646816	–	–	–
Lasiodiplodia venezuelensis	CBS 118739; CMW 13511; WAC 12539 ex-type	Wood of living *Acacia mangium*	Venezuela	S. Mohali	DQ103547	DQ377904	–	–	–
Macrophomina phaseolina	CBS 162.25	*Eucalyptus* sp.	Uganda	–	KF531826	DQ377905	–	–	–
	CBS 227.33	*Zea mays*	Palestine	–	KF531825	DQ377906	–	–	–
Marasasiomyces karoo	CBS 118718; IMI 186782 ex-type	Dead stems of *Eriocephalus* sp.	South Africa: Cape Province	W.F.O. Marasas	KF531828	DQ377939	KF531807	KF531808	KF531827
Mucoharknessia cortaderiae	CPC 19974 ex-type	Leaves of *Cortaderia selloana*	Argentina: Buenos Aires Province	F.E. Anderson	KM108374	KM108401	–	–	–
Mucoharknessia cortaderiae	CPC 22208	Leaves of *Cortaderia selloana*	Argentina: Buenos Aires Province	F.E. Anderson	KM108375	KM108402	–	–	–
Neodeightonia palmicola	MFLUCC 10-0823	*Caryota urens*	Thailand	J.K. Liu & R. Phookamsak	HQ199224	HQ199225	–	–	–
Neodeightonia phoenicum	CBS 122528 ex-type	*Phoenix* sp.	Spain: Catalonia	F. Garcia	KF766198	EU673261	–	–	–
Neodeightonia subglobosa	CBS 448.91 ex-type	Dead culms of *Bambusa arundinacea*	Sierra Leone	F.C. Deighton	KF766199	DQ377866	–	–	–
Neoscytalidium dimidiatum	CBS 145.78 ex-isotype	Sole of human foot	United Kingdom	C.K. Campbell	KF531816	DQ377922	–	–	–
	CBS 251.49; IMI 031449; UAMH 6803	*Juglans regia*	USA: California	–	KF531819	DQ377923	–	–	–
Phaeobotryon mamane	CPC 12264	*Sophora chrysophylla*	USA: Hawaii	W. Gams	EU673331	DQ377898	–	–	–
	CPC 12440	*Sophora chrysophylla*	USA: Hawaii	W. Gams	EU673332	EU673248	–	–	–
Saccharata proteae	CBS 115206; CPC 4378	*Protea* sp.	Australia	M.E. Palm	KF531812	GU301869	–	–	–

Sakireeta madreeya	CBS 532.76	Undetermined grass host	India: Madras	R.S. Mehrotra	KM108376	DQ377940	KM108427	–	–
Sphaeropsis citrigena	ICMP 16812 ex-type	Recently dead bark-covered twigs of *Citrus sinensis*	New Zealand: Northland	S.R. Pennycook, P.R. Johnston & B.C. Paulus	EU673328	EU673246	–	–	–
Sphaeropsis eucalypticola	CBS 133993; MFLUCC 11-0579 ex-type	Dead twig of *Eucalyptus* sp.	Thailand: Chiang Rai Province	M. Doilom	JX646802	JX646819	–	–	–
Sphaeropsis porosa	CBS 110496; CPC 5132 ex-type	*Vitis vinifera*	South Africa: Western Cape Province	J.M. van Niekerk	AY343379	DQ377894	–	–	–
Sphaeropsis visci	CBS 100163	Dead fallen twigs of *Viscum album*, under *Populus* sp.	Luxembourg	H.A. van der Aa	EU673324	DQ377870	–	–	–
Tiarosporella paludosa	CBS 114650; UPSC 3256	*Eleocharis palustris*	Sweden	K. & L. Holm	KM108377	KM108403	KM108428	KM108470	–
	CPC 22701 ex-epitype	*Trichophorum cespitosum* subsp. *germanicum*	Netherlands: Winterswijk	W. Quaedvlieg	KM108378	KM108404	–	KM108471	KM108450
	CPC 22702	*Trichophorum cespitosum* subsp. *germanicum*	Netherlands: Winterswijk	W. Quaedvlieg	KM108379	KM108405	–	KM108472	KM108451
Tiarosporella sp.	CBS 109699; LYN 451	Leaf spot on *Xanthorrhoea* sp.	Australia	C.F. Hill	KM108380	KM108406	KM108429	–	–

[1] CAP: AJL 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, Aukland, New Zealand; IMI: International Mycological Institute, CBI-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 nrDNA; LSU: large subunit (28S) of the nrRNA gene operon; TEF: partial translation elongation factor 1-alpha gene; TUB: partial beta-tubulin gene; SSU: small subunit (18S) of the nrRNA gene operon.

Discussion

Results from the present study revealed that the genus *Tiarosporella s.lat*. is actually poly- and paraphyletic. Tiarosporella-like taxa cluster in the Phacidiaceae, and Botryosphaeriaceae. Species of *Tiarosporella s.str.* belong to the Botryosphaeriaceae. Those species clustering in the Phacidiaceae (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 conidiogenous 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 conidiogenous 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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