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Families, genera, and species of *Botryosphaeriales*

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ABSTRACT

Members of *Botryosphaeriales* are ecologically diverse, but most commonly associated with leaf spots, fruit and root rots, die-back or cankers of diverse woody hosts. Based on morphology and DNA sequence data, the *Botryosphaeriales* have to date been shown to contain eight families, with an additional two, *Endomelanconiopsisaceae* (*Endomelanconiopsis*) and *Pseudofusicoccumaceae* (*Pseudofusicoccum*) being newly described in this study. Furthermore, *Oblongocollomyces* is introduced as new genus, while *Spencermartinsia* is reduced to synonymy under *Dothiorella*. Novel species include *Diplodia pyri* (*Pyrus* sp., the Netherlands), *Diplodia citricarpa* (*Citrus* sp., Iran), *Lasiodiplodia vitis* (*Vitis vinifera*, Italy), *L. sterculiae* (*Sterculia oblonga*, Germany), *Neofusicoccum pistaciarum* (*Pistacia vera*, USA), *N. buxi* (*Buxus sempervirens*, France), *N. stellenboschiana* (*Vitis vinifera*, South Africa), and *Saccharata hawaiiensis* (*Protea laurifolia*, Hawaii). New combinations are also proposed for *Camarosporium pistaciae* (associated with fruit rot of *Pistacia vera*) in *Neofusicoccum*, and *Sphaeria gallae* (associated with galls of *Quercus*) in *Diplodia*. The combination of large subunit of the nuclear ribosomal RNA gene (LSU)-*rpb2* proved effective at delineating taxa at family and generic level. Furthermore, *rpb2* also added additional resolution for species delimitation, in combination with ITS, *tef1* and *tub2*. In this study we analysed 499 isolates, and produce an expanded phylogenetic backbone for *Botryosphaeriales*, which will help to delimit novelties at species, genus and family level in future.

Introduction

Species of *Botryosphaeriales* are distributed worldwide, and occur on a wide range of different host plants. They have a diverse ecology, and can be saprobic, endophytic, or plant pathogenic (Slippers & Wingfield 2007; Jami *et al.* 2014; Slippers *et al.* 2014; Trakunyingcharoen *et al.* 2015; Crous *et al.* 2016), causing a range of disease symptoms including

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leaf spots, fruit and root rots, die-back or cankers (Phillips *et al.* 2013; Sarr *et al.* 2014; Trakunyingcharoen *et al.* 2014). In addition, some species are even known to cause opportunistic infections in humans (de Hoog *et al.* 2000).

The genus *Botryosphaeria* is based on *B. dothidea*, which was epitypified by Slippers *et al.* (2004), thereby fixing the generic application of the name. Schoch *et al.* (2006) subsequently introduced the order *Botryosphaeriales* (*Dothideomycetes*), which at the time contained a single family, *Botryosphaeriaceae*. In a further study Minnis *et al.* (2012) resolved the status of *Planistromellaceae* (*Kellermania*), showing it to also belong to this order, which was also confirmed by Liu *et al.* (2012), who delineated several additional unresolved lineages within *Botryosphaeriales*. To this extent Wikee *et al.* (2013) resurrected the *Phyllostictaceae* (*Phyllosticta*), while Slippers *et al.* (2013) introduced the new families *Aplosporellaceae* (*Aplosporella* and *Bagnisiella*), *Melanopsaceae* (*Melanops*), and *Saccharataceae* (*Saccharata*), and Wyka & Broders (2016) introduced *Septorioideaceae* (*Septorioides*).

Other than *Botryosphaeria*, several additional genera have over the years been recognised as belonging to *Botryophaeriaceae*. Liu *et al.* (2012) examined numerous generic type specimens, and based on morphology accepted 29 genera in the family. However, based on the species known from culture and supported by DNA data, Phillips *et al.* (2013) only recognised 17 genera and 110 species. The amendment of article 59 of the International Code of Nomenclature for algae, fungi and plants, and the One Fungus = One Name initiative (Wingfield *et al.* 2012; Crous *et al.* 2015) again saw several genera being merged, most notably *Botryosphaeria* (=*Fusicoccum*), *Kellermania* (*Planistroma*, *Panistromella*), *Phyllosticta* (=*Guignardia*), and *Sphaeropsis* (*Phaeobotryosphaeria*) (Crous *et al.* 2014, 2015a; Wijayawardene *et al.* 2014; Rossman *et al.* 2015). Furthermore, a revision of the *Tiarosporella* complex saw the number of genera rise to 22 within *Botryosphaeriaceae* (Crous *et al.* 2015c).

The first single-gene phylogeny with extended sampling led to the recognition of 10 botryosphaeria-like clades (Crous *et al.* 2006), which were later recognised as a family based on multi-gene data, separating the *Botryosphaeriaceae* (as *Botryosphaeriales*) from the *Pleosporales* and *Dothideales* (Schoch *et al.* 2006). The discovery of new families in this order has since relied on DNA phylogenetic data (Minnis *et al.* 2012; Slippers *et al.* 2013; Wikee *et al.* 2013; Wyka & Broders 2016), supported by morphological characteristics. However, the taxonomic status of several lineages remained unresolved at family, generic or species level.

The CBS-KNAW Fungal Biodiversity Centre (CBS) culture collection in Utrecht, The Netherlands, contains numerous *Botryosphaeriaceae* cultures, many of which were subject of past studies (e.g. Crous *et al.* 2006, 2015c; Phillips *et al.* 2013; Slippers *et al.* 2013, Sarr *et al.* 2014) or were deposited by other authors in the culture collection as part of their own manuscripts (e.g. Begoude *et al.* 2010; Jami *et al.* 2013; Linaldeddu *et al.* 2015, to list but a few). However, more than 100 cultures in the culture collection remain listed without a species epithet; the largest number of these only being listed as *Botryosphaeria* sp.

The main aim of the present study was to re-examine strains of the unidentified *Botryosphaeriales* in the CBS, and to resolve their taxonomy in light of current molecular-based knowledge in *Botryosphaeriales*. Furthermore, the current taxonomy of *Botryosphaeriales* is revisited by employing new genes with appropriate evolutionary rates, and finding morphological characters to support or refute the currently accepted classification.

Materials and methods

Isolates

Isolates used in this study were obtained from CBS, Utrecht, The Netherlands, which included available ex-type strains of described species (Supplementary Table S1). To induce sporulation, isolates were inoculated onto sterile pine needles and placed on 2 % water agar (PNA; Smith *et al.* 1996), potato dextrose agar (PDA), malt extract agar, oatmeal agar (OA; Crous *et al.* 2009b), and incubated at 25 °C under near-ultraviolet light for 2–4 wks.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from 7-d-old axenic cultures, grown on MEA at room temperature, using the UltraClean™ Microbial DNA isolation kit (Mo Bio Laboratories, California, USA) following the protocols provided by the manufacturer. Partial gene sequences were determined for the large subunit of the nuclear ribosomal RNA gene (LSU), DNA-directed RNA polymerase II second largest subunit (*rpb2*), the translation elongation factor 1-alpha (*tef1*), the internal transcribed spacer 1 and 2 including the intervening 5.8S nrDNA gene (ITS), and the β-tubulin gene (*tub2*) using the primers listed in Table 1. Amplification protocols followed Pavlic *et al.* (2009), Phillips *et al.* (2013) and Slippers *et al.* (2013). Amplicons were sequenced in both directions using a BigDye Terminator v. 3.1 Cycle Sequencing Kit (ThermoFisher Scientific) according to the manufacturer's instructions. A consensus sequence was manually generated for each set of forward and reverse sequences in MEGA v. 6 (Tamura *et al.* 2013). Additional sequences were obtained from GenBank using megaBLAST searches (accessions KX463943–KX465087; Supplementary Table S1).

Phylogenetic analyses

Based on the blast searches, strains were binned into generic groups and a subset was used to create an overview phylogeny. Sequences were aligned using MAFFT v. 7 (Katoh & Standley 2013) and refined manually after which the multiple alignments were concatenated following the combinations listed for each set in Table 2. The datasets were subjected to Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) analyses.

The ML analysis was performed using RAxML v. 8 (randomised accelerated (sic) ML for high performance computing; Stamatakis 2014) through the RAxML blackbox website (http://embnet.vital-it.ch/raxml-bb/) to obtain an additional measure of branch support. The option for the gamma model of rate heterogeneity was selected and the proportion of

Table 1 – Details of primer combinations used during this study for generic amplification and sequencing.

Locus	Primer name	Sequence 5′→3′	Tm (°C)	Reference
ITS	ITS5	GGA AGT AAA AGT CGT AAC AAG G	52	White et al. (1990)
	ITS4	TCC TCC GCT TAT TGA TAT GC	52	White et al. (1990)
LSU	LR0R	ACC CGC TGA ACT TAA GC	52	Rehner & Samuels (1994)
	LR5	TCC TGA GGG AAA CTT CG	52	Vilgalys & Hester (1990)
tef1	EF1-728F	CAT CGA GAA GTT CGA GAA GG	54	Carbone & Kohn (1999)
	EF1-986R	TAC TTG AAG GAA CCC TTA CC	54	Carbone & Kohn (1999)
tub2	Bt-2a	GGT AAC CAA ATC GGT GCT GCT TTC	52	Glass & Donaldson (1995)
	Bt-2b	ACC CTC AGT GTA GTG ACC CTT GGC	52	Glass & Donaldson (1995)
rpb2	RPB2-6F	TGG GGK WTG GTY TGY CCT GC	60 → 58 → 54	Liu et al. (1999)
	fRPB2-7cR	CCC ATR GCT TGY TTR CCC AT	60 → 58 → 54	Liu et al. (1999)
	RPB2bot6F	GGT AGC GAC GTC ACT CCC	60 → 58 → 54	Sakalidis et al. (2011)
	RPB2bot7R	GGA TGG ATC TCG CAA TGC G	60 → 58 → 54	Sakalidis et al. (2011)

Table 2 – Statistical information of the different analyses performed in this study.

Analysis	Substitution models used for Bayesian analysis/unique site patterns				
	LSU	*rpb2*	ITS	*tef1*	*tub2*
Overview	GTR+I+G/161	GTR+I+G/404	–	–	–
Diplodia	–	–	HKY+G/89	HKY+G/118	HKY+G/130
Dothiorella	–	–	GTR+I+G/102	GTR+G/207	GTR+I+G/125
Lasiodiplodia 3-gene	–	–	GTR+I/45	HKY+G/88	GTR+I/62
Lasiodiplodia 2-gene	–	–	HKY+G/50	HKY+I+G/107	–
Neofusicoccum 4-gene	–	GTR+I/140,	GTR+I+G/122	HKY+G/145	GTR+I/131
Neofusicoccum 2-gene	–	–	GTR+I+G/113	HKY+G/164	–
Phaeobotryon/Barriopsis	HKY+I/32	–	HKY+I/32	HKY+G/124	–
Saccharata	–	–	SYM+G/64	HKY+I+G/65	GTR+G/65
Tiarosporella-like	–	–	K80+I/55	HKY+I/199	GTR+I+G/155

Analysis	Statistics for the parsimony analyses				
	Number of strains (incl. outgroup(s)	Number of included characters	Number of parsimony-informative characters	Number of parsimony-uninformative	Number of constant sites
Overview	100	1450	460	122	868
Diplodia	90	1275	264	101	910
Dothiorella	71	1285	397	50	838
Lasiodiplodia 3-gene	28	1170	104	129	937
Lasiodiplodia 2-gene	72	733	114	54	565
Neofusicoccum 4-gene	60	1885	322	240	1323
Neofusicoccum 2-gene	199	847	268	51	528
Phaeobotryon/Barriopsis	10	1417	140	162	1115
Saccharata	15	1614	97	453	1064
Tiarosporella-like	19	1032	366	93	573

Analysis	Tree length	Consistency index (CI)	Retention index (RI)	Rescaled CI (RC)	Number of saved trees
Overview	2724	0.358	0.812	0.291	72
Diplodia	655	0.704	0.930	0.654	1000
Dothiorella	1202	0.606	0.923	0.560	1000
Lasiodiplodia 3-gene	385	0.706	0.735	0.520	2
Lasiodiplodia 2-gene	347	0.648	0.873	0.566	1000
Neofusicoccum 4-gene	1068	0.668	0.845	0.564	384
Neofusicoccum 2-gene	675	0.665	0.957	0.636	100
Phaeobotryon/Barriopsis	442	0.869	0.760	0.661	1
Saccharata	664	0.920	0.735	0.676	10
Tiarosporella-like	971	0.759	0.829	0.629	2

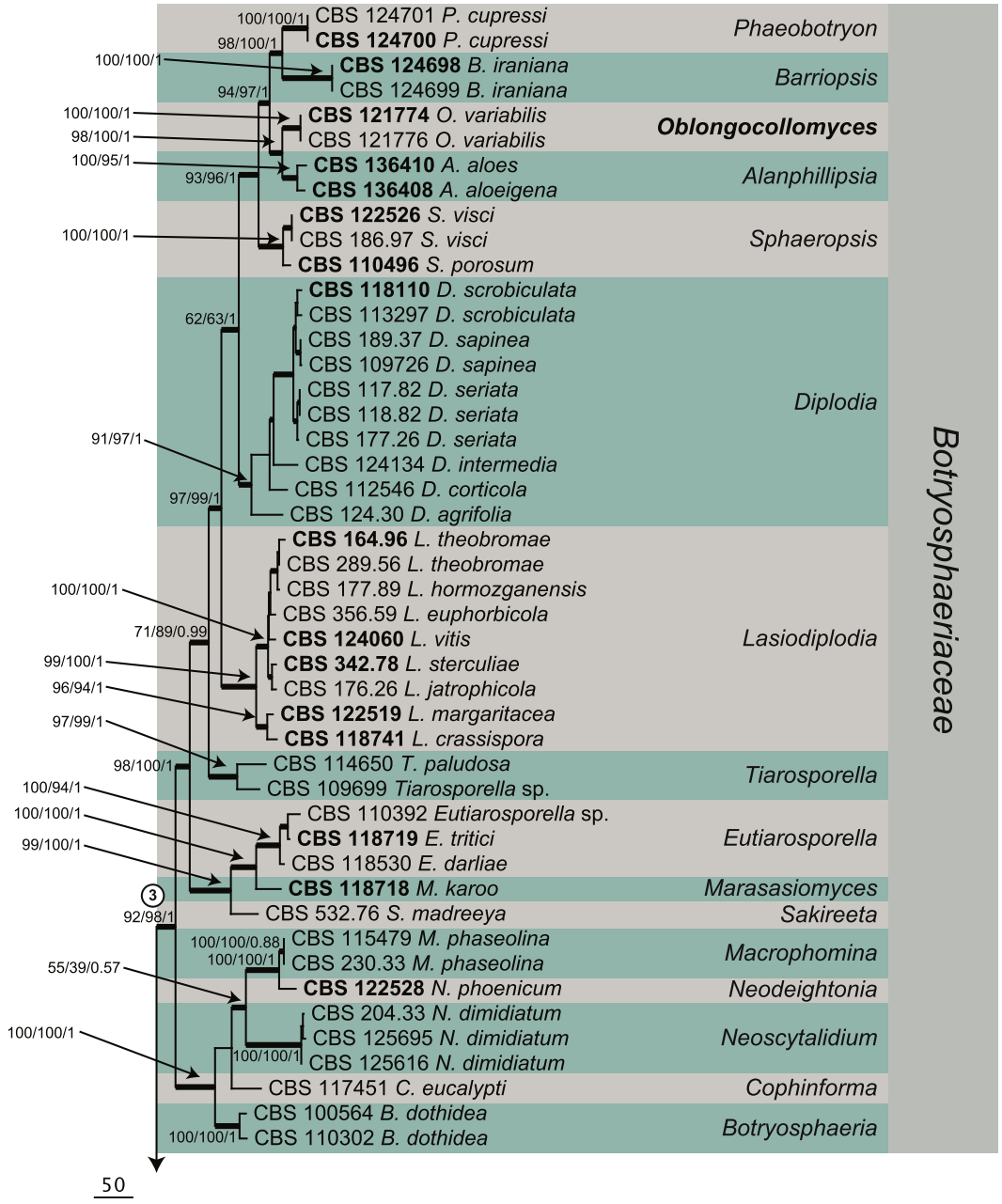


Fig 1 – The first of 72 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined LSU and *rpb2* sequence alignment of selected families in the *Botryosphaeriales*. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RAxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the deeper nodes or nodes representing distinct genera (MP-BS/ML-BS/PP). Ex-type strains and are indicated in bold font and genera and families are indicated to the right of the tree in blocks of different colours. The three possible family boundaries for the *Botryosphaeriaceae* are indicated by numbered white circles. Thickened lines represent those branches present in the parsimony strict consensus tree. Some branches were shortened to facilitate layout of the tree. The tree was rooted to *Phaeosphaeria ammophilae* (CBS 114595; *Pleosporales*, *Phaeosphaeriaceae*).

invariable sites was estimated by the software. The robustness of the analysis was evaluated by bootstrap support (MLBS) with the number of bootstrap replicates automatically determined by the software.

The MP analyses were performed in PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003) with phylogenetic relationships estimated by heuristic searches with 100 random taxon additions. The tree-bisection-

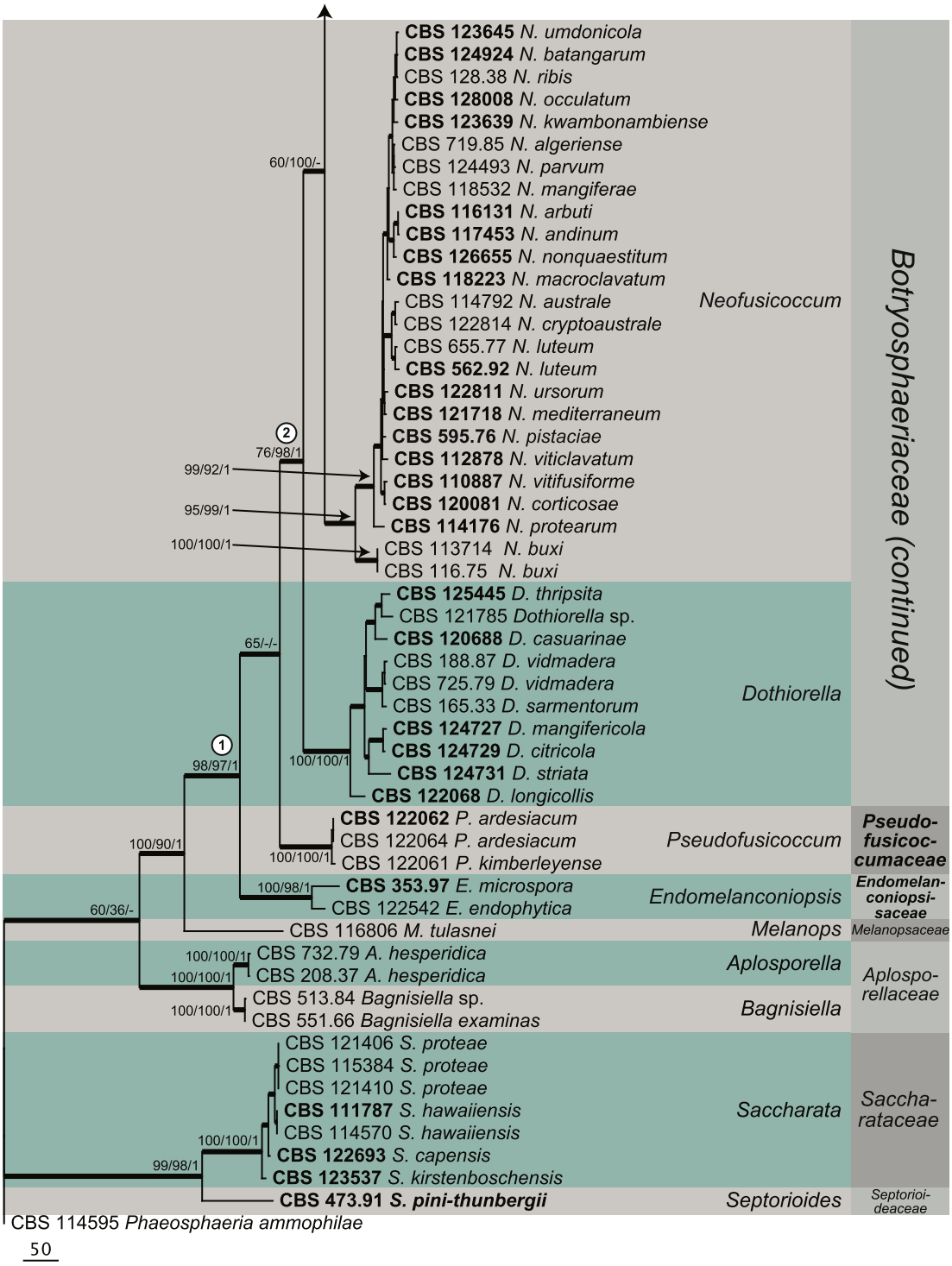


Fig 1 – (continued).

reconnection option was used, with the branch swapping option set to 'best trees' only. All characters were weighted equally and alignment gaps were treated as fifth state. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) (Table 2). Bootstrap analyses (MPBS, Hillis & Bull 1993) were based on 1000 replications.

Prior to the Bayesian analyses, MrModeltest (Nylander 2004) was used to determine evolutionary model for each partition (Table 2). The Bayesian analyses were performed using MrBayes v. 3.2.8 (Ronquist & Huelsenbeck 2003) with the Markov Chain Monte Carlo (MCMC) algorithm and two sets of four chains started in parallel from a random tree topology were employed with the heating parameter set at 0.3. The MCMC analysis ran until the average standard deviation of split frequencies came

below 0.01, with trees saved every 100 or 1000 generations, depending on the matrix size. The first 25 % of saved trees were discarded as the 'burn-in' phase and posterior probabilities (PP) determined from the remaining trees.

All resulting trees were printed using Geneious v. 5.5.4 (Kearse *et al.* 2012); layout of trees was done using Adobe Illustrator CS5. Novel sequences derived in this study were deposited in GenBank and the alignments and trees in TreeBASE (Study 20109; www.treebase.org/treebase/index.html).

Evaluation of loci for species resolution

The ability of loci to distinguish the species included in each alignment was evaluated as described by Gomes *et al.* (2013) and Alvarez *et al.* (2016). In short, neighbour joining (NJ) trees with the HKY85 substitution model were generated for each locus of the alignments used to generate Figs 1–8 and S1–S2. The trees were then manually evaluated for its ability to resolve species as distinct from one another. A species was only counted if it was distinct from its closest relatives and if the species clade contained all strains of that species included in the analysis. Species names were applied to the trees based on the phylogenetic analyses performed in the present study, but also based on prior studies where in some cases more, or other, loci were employed for species recognition than the ones used here. These are indicated by additional taxon names after the accession number in Figs 1–8 and S1–S2.

Morphological characteristics

Fungal structures were mounted in clear lactic acid (85 % v/v) and studied under a Nikon SMZ25 stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and a Nikon DS-Ri2 camera and software. Thirty observations were made of each taxonomically informative structure where possible, and for spores the 95 % percentiles are presented, with extremes given between brackets. Colony characters and pigment production were noted after 1 wk of growth on PDA, MEA and OA incubated at 25 °C in the dark. Colony colours (surface and reverse) were rated according to the colour chart of Rayner (1970). Reference specimens were deposited in the CBS fungarium, and nomenclature and descriptions of taxonomic novelties in MycoBank (Crous *et al.* 2004).

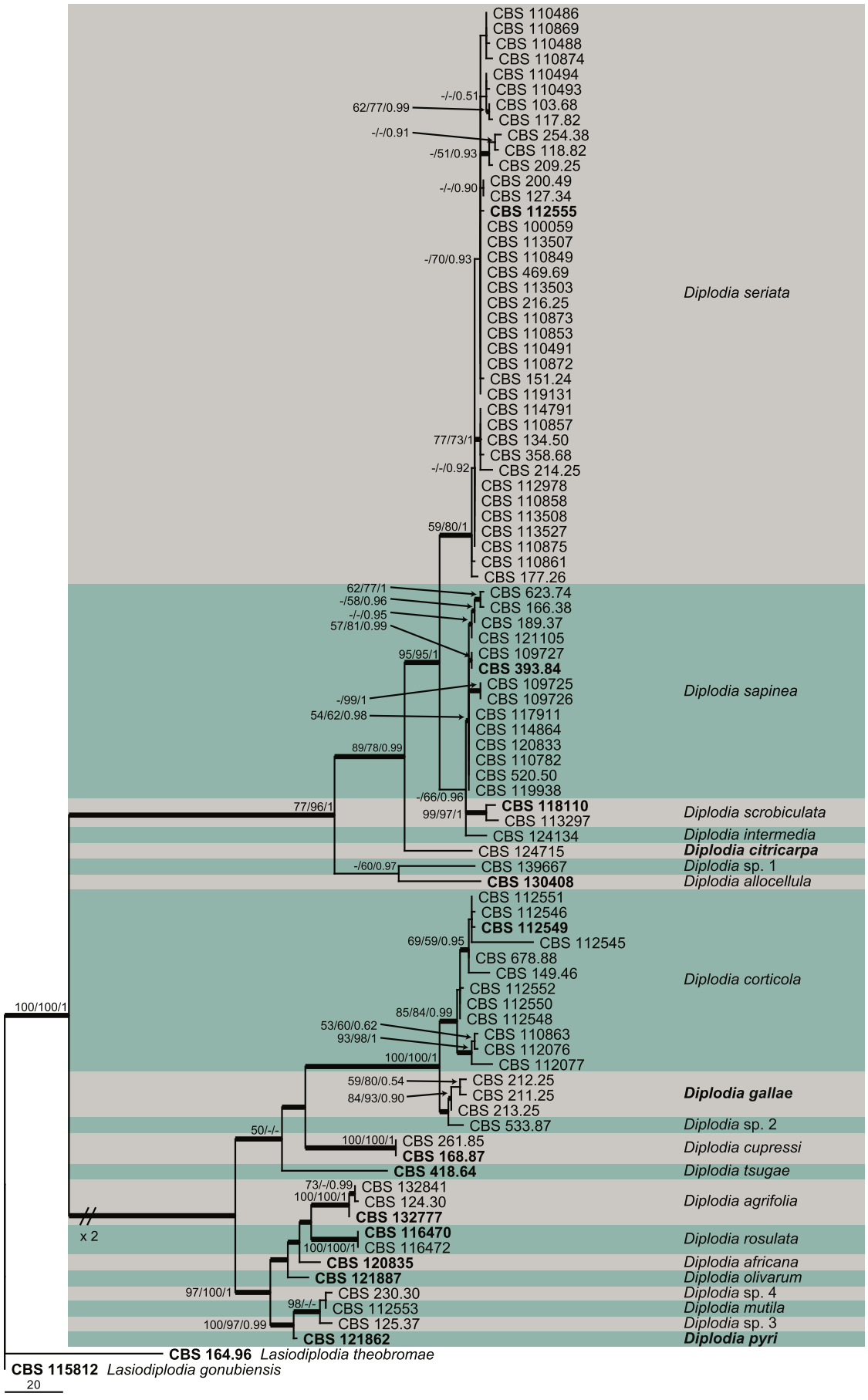
Results

Phylogenetic analyses

In total, eight multigene phylogenies (Figs 1–8) were generated, with an additional two unpublished phylogenies each containing a two-gene alignment of a larger set of isolates for *Lasiodiplodia* and *Neofusicoccum* (data not shown, see TreeBASE). Statistics and models used for the different datasets are shown in Table 2. The most parsimonious trees from each alignment are presented here, with the support values from the RAxML and Bayesian analyses plotted in addition at the nodes. A phylogeny of all the families and genera are provided in Fig 1 and revealed one novel genus, *Oblongocollomyces*, and two novel families, *Endomelanconiopsisaceae* (*Endomelanconiopsis*) and *Pseudofusicoccumaceae* (*Pseudofusicoccum*), which are described below. The remaining analyses treated *Diplodia* (Fig 2), *Dothiorella* (including *Spencermartinsia*; Fig 3), *Lasiodiplodia* (Fig 4), *Neofusicoccum* (Fig 5), *Phaeobotryon* and *Barriopsis* (Fig 6), *Saccharata* (Fig 7) and tiarosporella-like genera (Fig 8). The *Diplodia* phylogeny (Fig 2) revealed three taxonomic novelties which are treated below, and a further four isolates most likely representing novel species but which are not described in the present paper. Species of *Spencermartinsia* clustered inside *Dothiorella* (Fig 3) and therefore new combinations in *Dothiorella* are provided for those species in *Spencermartinsia*. The *Lasiodiplodia* phylogeny (Fig 4) revealed two taxonomic novelties which are treated below, and one additional isolate that most likely represents novel species but which will not be described in the present paper. The *Neofusicoccum* phylogeny (Fig 5) revealed three taxonomic novelties which are treated below, and a further 10 isolates most likely representing novel species but which are not described in the present paper. The *Saccharata* phylogeny (Fig 7) revealed one taxonomic novelty which is treated below, and a further two isolates most likely representing novel species but which are not described in the present paper. No novelties were found in the phylogenies of *Phaeobotryon* and *Barriopsis* (Fig 6) and tiarosporella-like genera (Fig 8). The potentially novel taxa that were not described in this study were sterile and we refrained from describing these as new based only on fixed nucleotide positions. We believe that species of *Botryosphaeriaceae* are common enough that fresh, sporulating cultures of these sterile cultures are bound to be recollected in the near future, allowing for them to be properly described and illustrated. In addition, the majority of these potentially novel taxa are represented by single lineages, and a larger sampling of these is required to confirm the fixed nucleotide positions used for a description based on DNA data alone.

Evaluation of loci for species resolution

The evaluation of the success of the individual loci to resolve species revealed that the most optimal locus is related to the genus in question, and also to some degree, the sampling within the genus (Table 3). In the NJ overview phylogeny (data not shown), *rpb2* was able to discriminate all genera included in the phylogeny, whereas LSU only managed to achieve 80 % success. This was mainly due to species of the genera *Diplodia*, *Lasiodiplodia*, and *Sphaeropsis* not clustering into monophyletic lineages but sometimes intermixing or forming separate lineages. For several genera, such as *Diplodia*, *Lasiodiplodia*, *Neofusicoccum* (2-gene alignment), and *Saccharata*, ITS and *tef1* did not perform exceptionally well on their own to resolve the species phylogenies and species recognition requires the use of an additional locus such as *tub2*. Interestingly, in the 4-gene *Neofusicoccum* alignment, ITS and *tef1* did not perform so poorly (85 % versus 69 % for ITS and 73 % versus 55 % for *tef1*, 4-gene versus 2-gene respectively). In this case, the sample size appears to influence resolution; in the 2-gene alignment a larger sampling per species was included, resulting in more interspecific diversity which decreases the intraspecific variation. However, in *Lasiodiplodia*


CBS 110486
CBS 110869
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CBS 110874
CBS 110494
CBS 110493
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CBS 117.82
CBS 254.38
CBS 118.82
CBS 209.25
CBS 200.49
CBS 127.34
CBS 112555
CBS 100059
CBS 113507
CBS 110849
CBS 469.69
CBS 113503
CBS 216.25
CBS 110873
CBS 110853
CBS 110491
CBS 110872
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CBS 119131
CBS 114791
CBS 110857
CBS 134.50
CBS 358.68
CBS 214.25
CBS 112978
CBS 110858
CBS 113508
CBS 113527
CBS 110875
CBS 110861
CBS 177.26
Diplodia seriata
CBS 623.74
CBS 166.38
CBS 189.37
CBS 121105
CBS 109727
CBS 393.84
CBS 109725
CBS 109726
CBS 117911
CBS 114864
CBS 120833
CBS 110782
CBS 520.50
CBS 119938
Diplodia sapinea
CBS 118110
CBS 113297
Diplodia scrobiculata
CBS 124134
Diplodia intermedia
CBS 124715
Diplodia citricarpa
CBS 139667
Diplodia sp. 1
CBS 130408
Diplodia allocellula
CBS 112551
CBS 112546
CBS 112549
CBS 112545
CBS 678.88
CBS 149.46
CBS 112552
CBS 112550
CBS 112548
CBS 110863
CBS 112076
CBS 112077
Diplodia corticola
CBS 212.25
CBS 211.25
CBS 213.25
Diplodia gallae
CBS 533.87
Diplodia sp. 2
CBS 261.85
CBS 168.87
Diplodia cupressi
CBS 418.64
Diplodia tsugae
CBS 132841
CBS 124.30
CBS 132777
Diplodia agrifolia
CBS 116470
CBS 116472
Diplodia rosulata
CBS 120835
Diplodia africana
CBS 121887
Diplodia olivarum
CBS 230.30
CBS 112553
Diplodia sp. 4
Diplodia mutila
CBS 125.37
Diplodia sp. 3
CBS 121862
Diplodia pyri
CBS 164.96 Lasiodiplodia theobromae
CBS 115812 Lasiodiplodia gonubiensis
20
x 2
-/-/0.51
62/77/0.99
-/-/0.91
-/51/0.93
-/-/0.90
-/70/0.93
77/73/1
-/-/0.92
59/80/1
62/77/1
-/58/0.96
-/-/0.95
57/81/0.99
95/95/1
-/99/1
54/62/0.98
89/78/0.99
-/66/0.96
77/96/1
99/97/1
-/60/0.97
69/59/0.95
85/84/0.99
53/60/0.62
93/98/1
100/100/1
100/100/1
59/80/0.54
84/93/0.90
50/-/-
100/100/1
73/-/0.99
100/100/1
100/100/1
97/100/1
98/-/-
100/97/0.99

this effect was the strongest in the ITS analysis where the smaller sampling of the 3-gene alignment allowed for 50 % species recognition versus 25 % in the 2-gene alignment, whereas the success rate was identical for *tef1* between the two alignments. This implies that the intraspecific variation for this locus is lower in *Lasiodiplodia* compared to the intraspecific variation for ITS. In *Dothiorella*, both ITS and *tef1* performed quite well, and *tub2* less so. *Saccharata* is one of the few genera where none of the loci performed well on their own; species in this genus are therefore best resolved based on a combined phylogeny rather than on a phylogeny derived from any single locus. Only *S. capensis* and *S. kirstenboschensis* were resolved by all individual loci, while isolates of *Saccharata* sp. 1, *S. hawaiiensis* and *S. proteae* were intermingled in both the ITS and *tef1* NJ phylogenies (data not shown). For the remaining alignments, almost all loci were highly successful in resolving species and genera. The only exceptions were that ITS failed to resolve *Eutiarosporella africana* and *E. tritici* in the tiarosporella-like alignment and that *tub2* could not properly resolve the generic relationships. For example *Marasasiomyces* clustered inside *Eutiarosporella,* and the two species of *Tiarosporella*, although separate from the other included species in the alignment, did not form a monophyletic lineage.

Taxonomy

Based on the phylogenetic analyses conducted in this study, several new families, genera and species were delineated. These morphological descriptions are provided below.

Botryosphaeriaceae Theiss. & Syd., Ann. Mycol. 16: 16. 1918.
Type genus: *Botryosphaeria* Ces. & De Not.

Diplodia pyri Tao Yang & Crous, *sp. nov.* – MycoBank MB817633; Fig 9.
Etymology: Name refers to the host genus from which it was isolated, *Pyrus*.

Conidiomata pycnidial, stromatic, globose, immersed, becoming erumpent, separate, dark brown to black, unilocular, up to 300 μm diam, with central papillate neck up to 400 μm long, and 150 μm diam; wall of 6–8 layers of dark brown, thick-walled *textura angularis*. *Ostiole* central, circular, 50–70 μm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lageniform to subcylindrical, proliferating several times percurrently at apex, discrete, indeterminate, hyaline, smooth, arising from the inner wall of the locule, 7–15 × 3–6 μm. *Conidia* ellipsoid to subclavate, straight to slightly curved, at first aseptate, hyaline, granular, smooth, becoming 1 (–2)-euseptate, walls 1–1.5 μm diam, outer surface of wall appearing pitted, apex obtuse, base truncate, scar 3–4 μm diam, (21–)24–28 (–35) × (11–)13–15 (–18) μm.

Culture characteristics: Colonies covering dish in 7 d, with cottony aerial mycelium. Colonies surface on MEA glaucous grey, reverse dark olivaceous; on PDA surface and reverse grey olivaceous; on OA surface grey olivaceous.
Material examined: **The Netherlands**: Wijdenes, on *Pyrus* sp., collector and date unknown, (holotype CBS H-22718, culture ex-type CBS 121862 = PD 03708098).
Notes: *Diplodia pyri* has larger conidia than that of *D. pyricola* (on *Pyrus communis*, Chile; conidia ellipsoid to subovate, 1-septate, 22–24 × 10–12 μm; Spegazzini 1921). It is also easily distinguished from the hyaline conidia of *Dothiorella pyricola* (on *Pyrus* sp., Pakistan, conidia cylindrical-fusoid, 15.5–20 × 4.5 μm) and *Dothiorella pyri* (on *Pyrus communis*, Poland, conidia ellipsoid to ovate, 11–15 × 7–8.5 μm) (Aderhold 1905; Ahmad 1971).

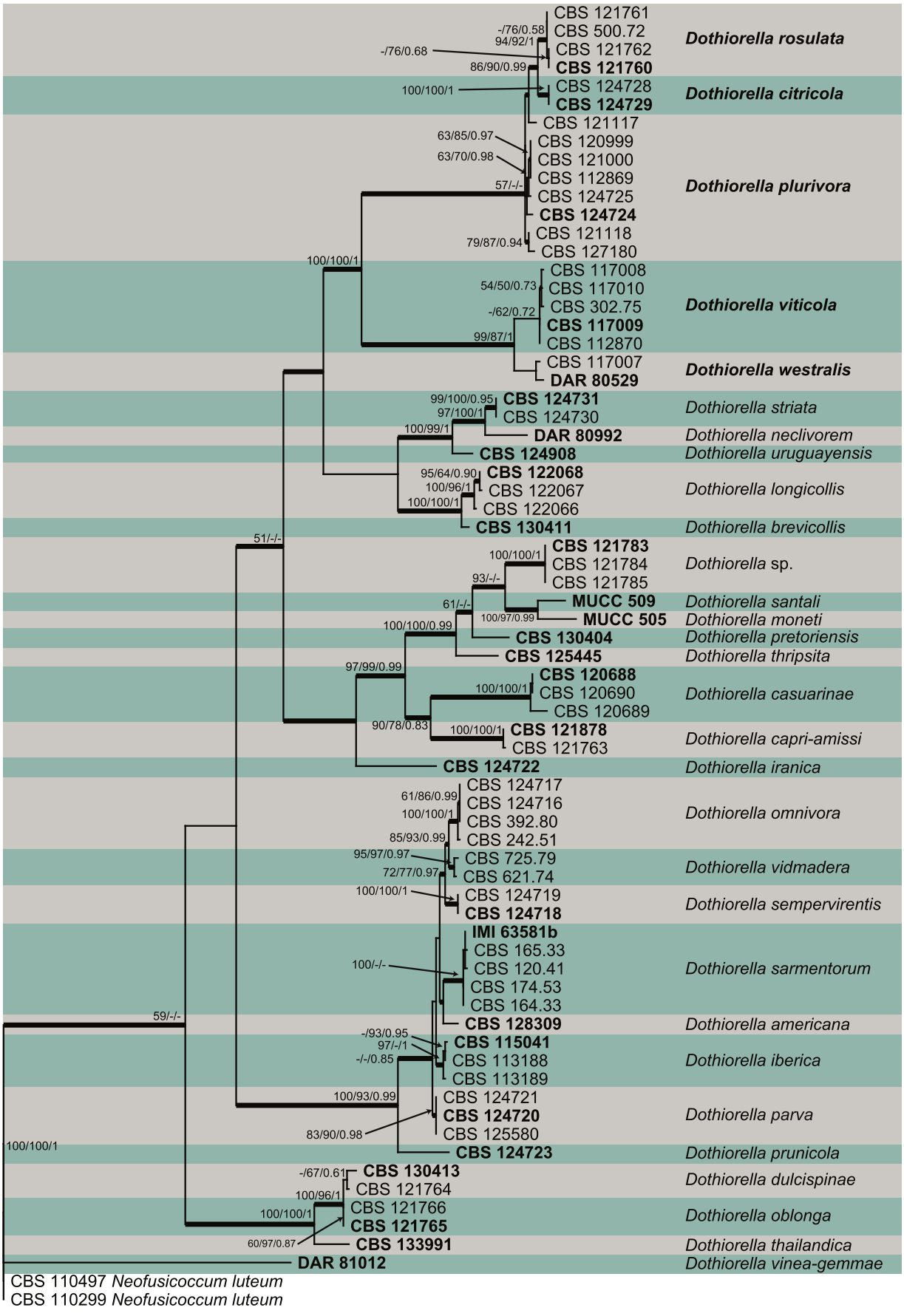
Diplodia citricarpa Abdollahz. & Crous, *sp. nov.* – MycoBank MB817634; Fig 10.
Etymology: Name refers to the host genus from which it was isolated, *Citrus*.

Conidiomata on PNA pycnidial, globose, erumpent, separate, dark brown to black, unilocular, up to 250 μm diam, with central ostiole; wall of 3–6 layers of dark brown, thick-walled *textura angularis*. *Paraphyses* not observed. *Conidiophores* reduced to conidiogenous cells or with a supporting cell. *Conidiogenous cells* subcylindrical, proliferating several times percurrently at apex, discrete, indeterminate, hyaline, smooth, arising from the inner wall of the locule, 6–15 × 4–5 μm. *Conidia* solitary, subcylindrical, at first aseptate, becoming 1-septate with age, granular, medium brown, finely verruculose, apex obtuse, base truncate, scar 3–4 μm diam, (19–)22–25 (–27) × 9 (–10) μm.

Culture characteristics: Colonies covering dish in 2wk, with fluffy aerial mycelium. Colonies on MEA, PDA, and OA surface dark mouse grey, reverse fuscous black.
Material examined. **Iran**: Hormozgan, Minab, Hajikhademi, on twigs of *Citrus* sp., 3 Mar. 2007, J. Abdollahzadeh & A. Javadi (holotype IRAN 14274F, isotype CBS H-22719, culture ex-type CBS 124715 = CJA 131 = IRAN 1578C); CBS 124714 = IRAN 1510C.
Notes: *Diplodia citricarpa* represents a novel species on *Citrus*, with larger conidia than that of *D. citri* (*Citrus*, Italy, conidia ellipsoid, 1-septate, 18–21 × 8–10 μm; Saccardo 1884), and *D. citricola* (*Citrus*, Australia, conidia elongated ellipsoid, 1-septate, 6–8 × 2.5–3.5 μm; McAlpine 1899). Finally, conidia are also smaller than those of *D. citrina* (*Citrus*, India, conidia ellipsoid, 1-septate, 20–25 × 10–13 μm; Sydow *et al.* 1916).

Diplodia gallae (Schwein.) Crous, *comb. nov.* – MycoBank MB817673.

Fig 2 – The first of 1000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined *tef1*, ITS and *tub2* sequence alignments of *Diplodia*. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the nodes (MP-BS/ML-BS/PP). Ex-type strains and taxonomic novelties are indicated in bold font and the species are delimited with coloured blocks. Thickened lines represent those branches present in the parsimony strict consensus tree. One branch was shortened to facilitate layout of the tree. The tree was rooted to *Lasiodiplodia gonubiensis* and *L. theobromae* (CBS 115812, CBS 164.96).


CBS 121761
-/76/0.58 CBS 500.72
94/92/1 CBS 121762
-/76/0.68 **CBS 121760**
86/90/0.99 **Dothiorella rosulata**
100/100/1 CBS 124728
CBS 124729 **Dothiorella citricola**
CBS 121117
63/85/0.97 CBS 120999
63/70/0.98 CBS 121000
CBS 112869
57/-/- CBS 124725
CBS 124724
CBS 121118
79/87/0.94 CBS 127180 **Dothiorella plurivora**
100/100/1
CBS 117008
54/50/0.73 CBS 117010
CBS 302.75
-/62/0.72 **CBS 117009**
99/87/1 CBS 112870 **Dothiorella viticola**
CBS 117007
DAR 80529 **Dothiorella westralis**
99/100/0.95 **CBS 124731**
97/100/1 CBS 124730 *Dothiorella striata*
100/99/1 **DAR 80992** *Dothiorella neclivorem*
CBS 124908 *Dothiorella uruguayensis*
95/64/0.90 **CBS 122068**
100/96/1 CBS 122067
100/100/1 CBS 122066 *Dothiorella longicollis*
CBS 130411 *Dothiorella brevicollis*
51/-/-
100/100/1 **CBS 121783**
CBS 121784
93/-/- CBS 121785 *Dothiorella* sp.
61/-/- **MUCC 509** *Dothiorella santali*
100/97/0.99 **MUCC 505** *Dothiorella moneti*
100/100/0.99 **CBS 130404** *Dothiorella pretoriensis*
CBS 125445 *Dothiorella thripsita*
97/99/0.99
100/100/1 **CBS 120688**
CBS 120690
CBS 120689 *Dothiorella casuarinae*
90/78/0.83
100/100/1 **CBS 121878**
CBS 121763 *Dothiorella capri-amissi*
CBS 124722 *Dothiorella iranica*
CBS 124717
61/86/0.99 CBS 124716
100/100/1 CBS 392.80
85/93/0.99 CBS 242.51 *Dothiorella omnivora*
95/97/0.97 CBS 725.79
72/77/0.97 CBS 621.74 *Dothiorella vidmadera*
100/100/1 CBS 124719
CBS 124718 *Dothiorella sempervirentis*
IMI 63581b
CBS 165.33
100/-/- CBS 120.41
CBS 174.53
CBS 164.33 *Dothiorella sarmentorum*
59/-/-
CBS 128309 *Dothiorella americana*
-/93/0.95 **CBS 115041**
97/-/1
-/-/0.85 CBS 113188
CBS 113189 *Dothiorella iberica*
100/93/0.99 CBS 124721
CBS 124720
83/90/0.98 CBS 125580 *Dothiorella parva*
100/100/1
CBS 124723 *Dothiorella prunicola*
-/67/0.61 **CBS 130413**
100/96/1 CBS 121764 *Dothiorella dulcispinae*
100/100/1 CBS 121766
CBS 121765 *Dothiorella oblonga*
60/97/0.87 **CBS 133991** *Dothiorella thailandica*
DAR 81012 *Dothiorella vinea-gemmae*
CBS 110497 *Neofusicoccum luteum*
CBS 110299 *Neofusicoccum luteum*
20

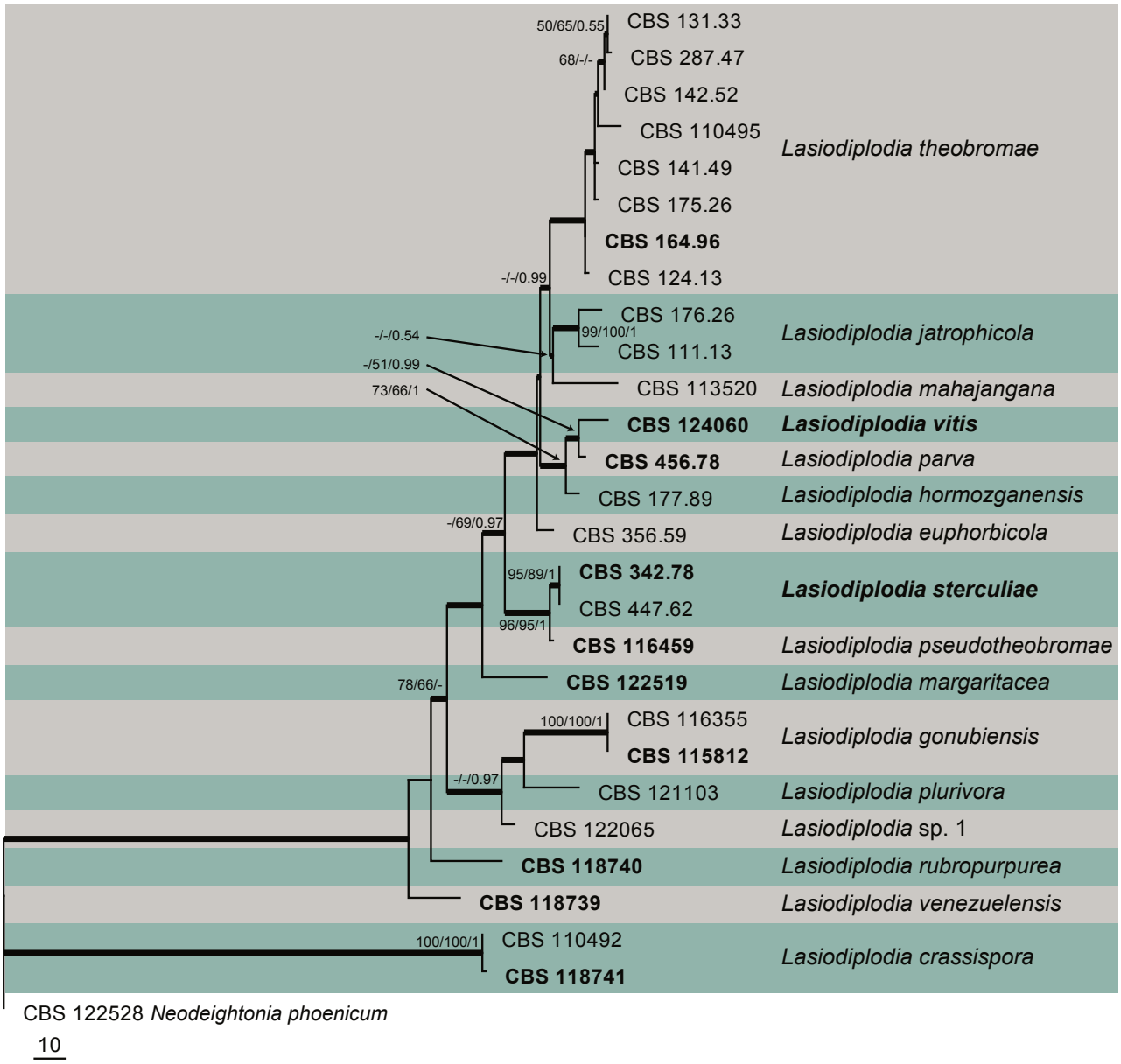


Fig 4 – The first of two equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined *tub2*, *tef1* and ITS sequence alignment of *Lasiodiplodia*. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the nodes (MP-BS/ML-BS/PP). Ex-type strains and taxonomic novelties are indicated in bold font and the species are delimited with coloured blocks. Thickened lines represent those branches present in the parsimony strict consensus tree. The tree was rooted to *Neodeightonia phoenicum* (CBS 122528).

Basionym: *Sphaeria gallae* Schwein., *Trans. Am. phil. Soc.*, New Series **4**(2): 207 (1832) [1834].
Synonyms: *Macroplodia gallae* (Schwein.) Kuntze, *Revis. gen. pl.* (Leipzig) **3**(2): 492 (1898).
Aplosporella gallae (Schwein.) Petr. [as '*Aplosporella*'], *Hedwigia* **65**: 273 (1925).
Botryodiplodia gallae (Schwein.) Petr. & Syd., Die Gattung der Pyrenomyzeten, Sphaeropsideen und Melanconieen: 152 (1926).

Fig 3 – The first of 1000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined *tef1*, ITS and *tub2* sequence alignment of *Dothiorella*. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the deeper nodes or nodes representing distinct genera (MP-BS/ML-BS/PP). Ex-type strains and taxonomic novelties are indicated in bold font and the species are delimited with coloured blocks. Thickened lines represent those branches present in the parsimony strict consensus tree. The tree was rooted to *Neofusicoccum luteum* (CBS 110299, CBS 110497).

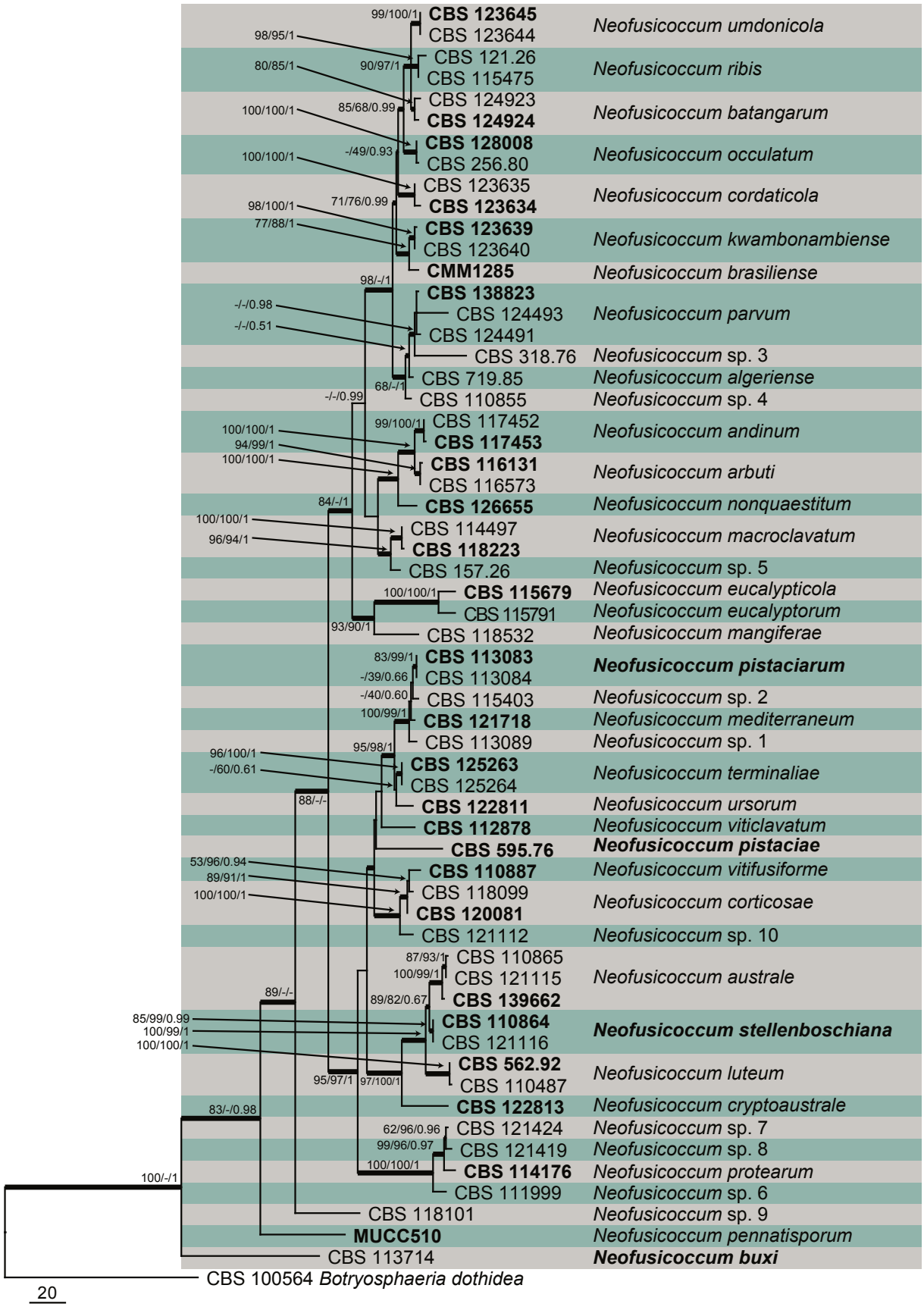


Fig 5 – The first of 384 equally most parsimonious trees obtained from a heuristic search with 25 random taxon additions of the combined *tef1*, ITS, *rpb2*, and *tub2* sequence alignment of *Neofusicoccum*. The scale bar represents the number of changes

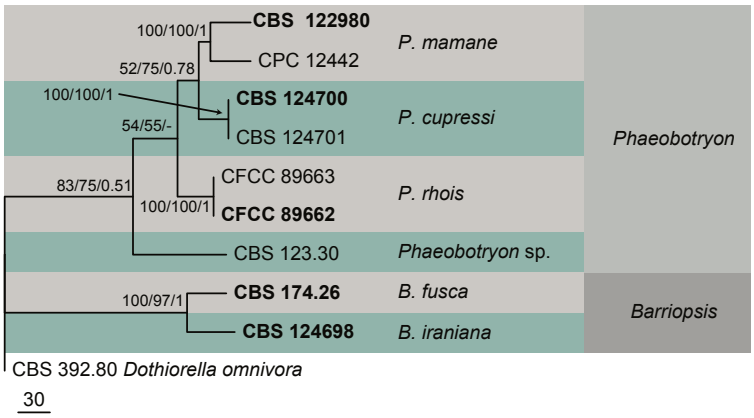


Fig 6 – The single most parsimonious tree obtained from a heuristic search with 100 random taxon additions of the combined LSU, *tef1* and ITS sequence alignment of *Phaeobotryon* and *Barriopsis*. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the nodes (MP-BS/ML-BS/PP). Ex-type strains are indicated in bold font and the species are delimited with coloured blocks. Genera are shown to the right of the tree in blocks of different colours. The tree was rooted to *Involcriomyces ignotus* (CBS392.80).

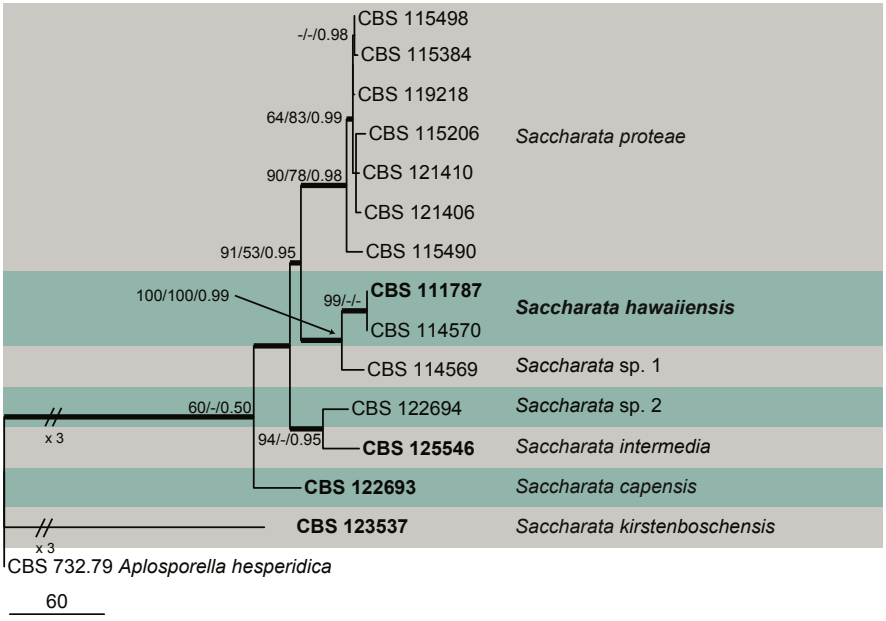


Fig 7 – One of 10 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined *tef1*, ITS, and *rpb2* sequence alignment of *Saccharata*. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the nodes (MP-BS/ML-BS/PP). Ex-type strains and taxonomic novelties are indicated in bold font and the species are delimited with coloured blocks. Thickened lines represent those branches present in the parsimony strict consensus tree. The tree was rooted to *Aplosporella* sp. (CBS 732.79).

and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the nodes (MP-BS/ML-BS/PP). Ex-type strains and taxonomic novelties are indicated in bold font and the species are delimited with coloured blocks. Thickened lines represent those branches present in the parsimony strict consensus tree. The tree was rooted to *Botryosphaeria dothidea* (CBS 100564).

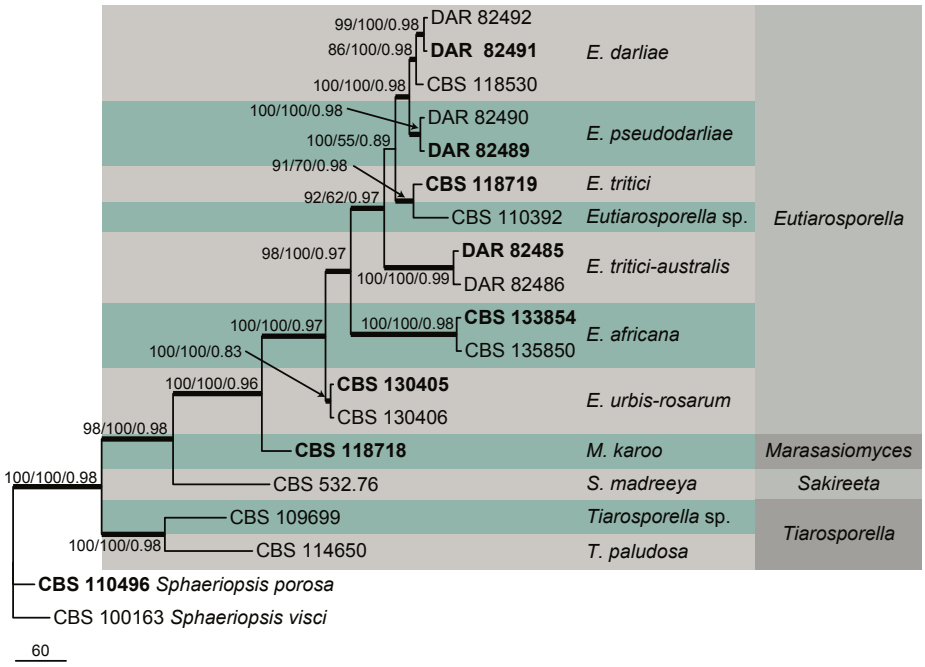


Fig 8 – One of two equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined *tef1*, ITS, and *tub2* sequence alignment of the tiarosporella-like group of genera. The scale bar represents the number of changes and bootstrap support values of MP (MP-BS) and RaxML analysis (ML-BS) and Bayesian posterior probability values (PP) are shown at the nodes (MP-BS/ML-BS/PP). Ex-type strains are indicated in bold font and the species are delimited with coloured blocks. Thickened lines represent those branches present in the parsimony strict consensus tree. Genera are shown to the right of the tree in blocks of different colours. The tree was rooted to *Sphaeropsis visci* and *Sphaeropsis porosa* (CBS 100163 and CBS 110496, respectively).

Sphaeropsis gallae (Schwein.) A.W. Archer, *Annls mycol.* **25**: 37 (1927).

Additional synonyms listed in Petrak & Sydow (1927).

Material examined: **Unknown**: on galls of *Quercus* sp., A.W. Archer, CBS 212.25, CBS 211.25, CBS 213.25.

Notes: *Sphaeria gallae* was described by D. von Schweinitz to name a fungus associated with galls on *Quercus* in the USA. The fungus was subsequently placed in several different genera, namely *Aplosporella*, *Botryodiplodia*, *Macroplodia*, and *Sphaeropsis*, all of which suggest it has dark conidia, which correlate with the strains clustering in *Diplodia*. The three cultures used by A.W. Archer (presumably from the USA) to introduce the combination in *Sphaeropsis*, were treated here, but unfortunately, all three (deposited in CBS in 1925) remained sterile. Nevertheless, a new combination is required for this fungus in *Diplodia*. This pathogen has in the past been referred to in literature under this name as *D. gallae* (Schwein.) Cooke (Wollenweber & Hochapfel 1941), although as far as we could establish, the combination has never been officially published.

Table 3 – Summary of the number of species/genera distinguished per locus for each alignment used in this study.

Analysis	LSU	*rpb2*[a]	ITS	*tef1*	*tub2*
Overview – genera	80 % (20/25)	100 % (25/25)	–	–	–
Overview – species	59 % (46/78)	91 % (71/78)	–	–	–
Diplodia	–	–	55 % (11/20)	65 % (13/20)	70 % (14/20)
Dothiorella	–	–	83 % (25/30)	93 % (28/30)	67 % (20/30)
Lasiodiplodia 3-gene	–	–	50 % (8/16)	56 % (9/16)	88 % (14/16)
Lasiodiplodia 2-gene	–	–	25 % (8/32)	56 % (18/32)	–
Neofusicoccum 4-gene	–	58 % (21/36)	85 % (35/41)	73 % (30/41)	63 % (26/41)
Neofusicoccum 2-gene	–	–	69 % (29/42)	55 % (23/42)	–
Phaeobotryon/Barriopsis – genera	50 % (1/2)	–	100 % (2/2)	100 % (2/2)	–
Phaeobotryon/Barriopsis – species	100 % (6/6)	–	100 % (6/6)	100 % (6/6)	–
Saccharata	–	43 % (3/7)	57 % (4/7)	57 % (4/7)	–
Tiarosporella-like – genera	–	–	82 % (9/11)	100 % (11/11)	100 % (11/11)
Tiarosporella-like – species	–	–	100 % (4/4)	100 % (4/4)	25 % (1/4)

a Not all isolates of *Neofusicoccum* amplified for this locus.

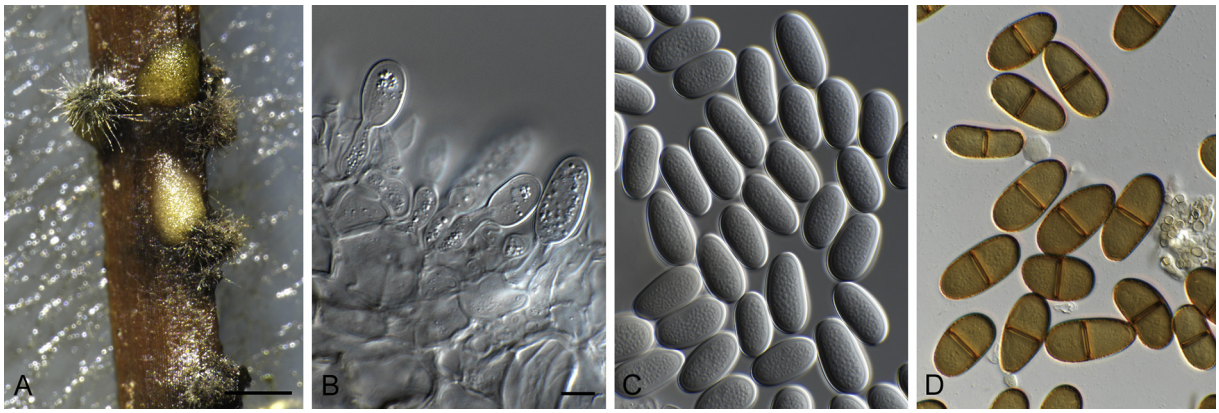


Fig 9 – *Diplodia pyri* (CBS 121862). (A). Colony sporulating on PNA. (B). Conidiogenous cells. (C). Young, hyaline conidia. (D). Mature, brown, 1-septate conidia. Scale bars: A = 300 μm, all others = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Dothiorella

The genus *Dothiorella* was resurrected by Phillips *et al.* (2005) to accommodate species with conidia that turn brown while still attached to their conidiogenous cells. Furthermore, Phillips *et al.* (2008) introduced *Spencermartinsia* to accommodate dothiorella-like species with apiculate ascospores. This decision was supported by a phylogeny based on ITS and *tef1*, and a rather limited taxon sampling (Phillips *et al.* 2013). Slippers *et al.* (2013) employed a six-gene phylogeny and more isolates, but found that additional loci and isolates could no longer separate *Dothiorella* from *Spencermartinsia*. Likewise in the present study, we found that a three-gene phylogeny again included *Spencermartinsia* in *Dothiorella*. From these results we conclude that apiculate ascospores, which evolved in several genera in the family (see Phillips *et al.* 2013) is not a reliable feature at generic level, and that these clades are best treated as a single genus, *Dothiorella*.

Dothiorella citricola (A.J.L. Phillips & Abdollahz.) Tao Yang & Crous, *comb. nov.* – MycoBank MB817674.
Basionym: *Spencermartinsia citricola* A.J.L. Phillips & Abdollahz., *Persoonia* **32**: 7 (2014).
Description and illustration: Abdollahzadeh *et al.* (2014).

Dothiorella mangifericola Tao Yang & Crous, *nom. nov.* – MycoBank MB817675.
Synonym: *Spencermartinsia mangiferae* Abdollahz. *et al.*, Persoonia **32**: 9 (2014).
Description and illustration: Abdollahzadeh *et al.* (2014).
Notes: Because the name *Dothiorella mangiferae* is already occupied, a new name is proposed for this species.

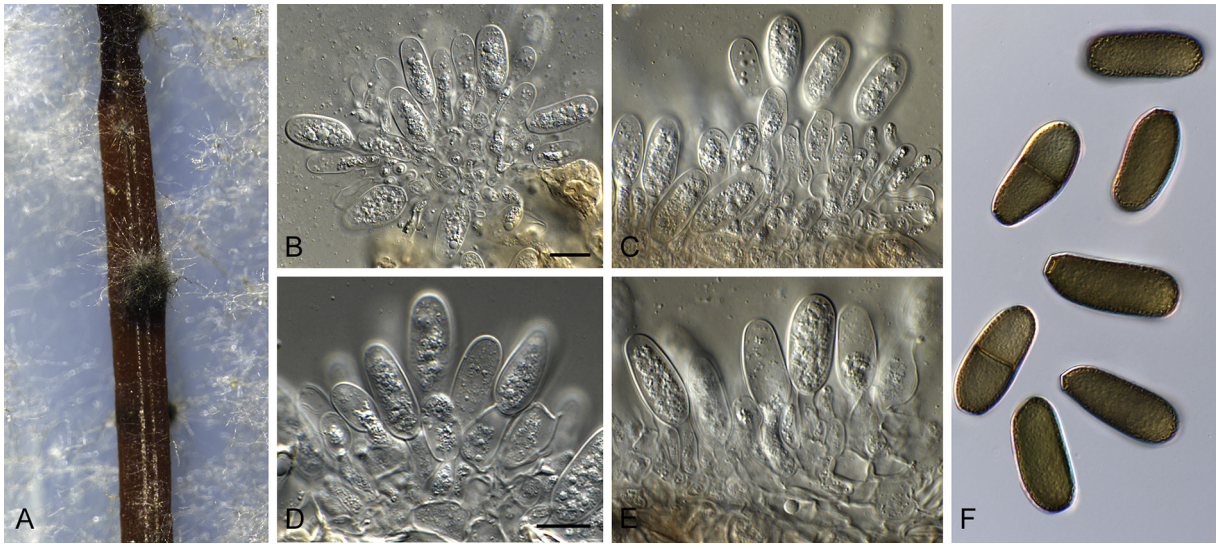


Fig 10 – *Diplodia citricarpa* (CBS 124715). (A). Colony sporulating on PNA. (B–E). Conidiogenous cells. (F). Mature, brown, 1-septate conidia. Scale bars: A = 250 μm, all others = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Dothiorella plurivora (Abdollahz. *et al.*) Tao Yang & Crous, *comb. nov.* – MycoBank MB817676.
Basionym: *Spencermartinsia plurivora* Abdollahz. *et al.*, Persoonia **32**: 9 (2014).
Description and illustration: Abdollahzadeh *et al.* (2014).

Dothiorella rosulata (F.J.J. Van der Walt *et al.*) Tao Yang & Crous, *comb. nov.* – MycoBank MB817677.
Basionym: *Spencermartinsia rosulata* F.J.J. Van der Walt *et al.*, Persoonia 33: 164 (2014).
Description and illustration: Slippers *et al.* (2014).

Dothiorella viticola A.J.L. Phillips & J. Luque, *Mycologia* **97**: 1118 (2006) [2005].
Synonyms: *Botryosphaeria viticola* A.J.L. Phillips & J. Luque, *Mycologia* **97**: 1118 (2006) [2005].
Spencermartinsia viticola (A.J.L. Phillips & J. Luque) A.J.L. Phillips *et al.*, *Persoonia* **21**: 51 (2008).
Description and illustration: Luque *et al.* (2005).

Dothiorella westralis (W.M. Pitt *et al.*) Tao Yang & Crous, *comb. nov.* – MycoBank MB817678.
Basionym: *Spencermartinsia westralis* (as westrale) W.M. Pitt *et al.*, Australas. Pl. Path. **44**: 48 (2015).
Description and illustration: Pitt *et al.* (2015).

Lasiodiplodia vitis Tao Yang & Crous, *sp. nov.* – MycoBank MB817635; Fig 11.
Etymology. Name refers to the host genus from which it was isolated, *Vitis*.

Conidiomata stromatic, formed on PNA in culture, uniloculate, up to 400 μm diam, dark brown to black; wall of 6–10 layers of brown *textura angularis*, immersed in the host tissue, becoming erumpent when mature, developing a central papillate neck up to 200 μm long, with ostiole 50–70 μm diam. *Paraphyses* intermingled among conidiogenous cells, hyaline, cylindrical, aseptate, unbranched, ends obtuse, up to 60 μm long, 2–3 μm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, ampulliform to subcylindrical, proliferating percurrently to form 1–3 closely spaced apical annellations, 5–15 × 5–8 μm. *Conidia* ellipsoidal, apex obtuse, base truncate, scar 4–5 μm diam, widest at the middle or upper third, thick-walled (2 μm diam), initially hyaline, granular and aseptate and remaining so for a long time, becoming 1-septate and dark brown after release from the conidiomata, with melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia, (25–)26–28 (–32) × (12–)15–16 (–17) μm.

Culture characteristics: Colonies covering dish in 7 d, with cottony aerial mycelium. Colonies on MEA, surface glaucous grey, reverse dark olivaceous; on PDA surface and reverse grey olivaceous; on OA surface olivaceous buff.
Material examined: **Italy**: Sicily, from canker symptoms of *Vitis vinifera*, date unknown, S. Burruano (holotype CBS H-22720, culture ex-type CBS 124060).
Notes: Rodríguez-Gálvez *et al.* (2017) present data to distinguish 15 different species of *Lasiodiplodia*. *Lasiodiplodia vitis*, which represents a novel species on *Vitis vinifera* in Italy, is clearly distinct from the taxa presently recognised on this host (Van Niekerk *et al.* 2006; Phillips *et al.* 2013), as most reports have been attributed to *L. theobromae* in the past (Burruano *et al.* 2008).

Lasiodiplodia sterculiae Tao Yang & Crous, *sp. nov.* – MycoBank MB817636; Fig 12.
Etymology: Name refers to the host genus from which it was isolated, *Sterculia*.

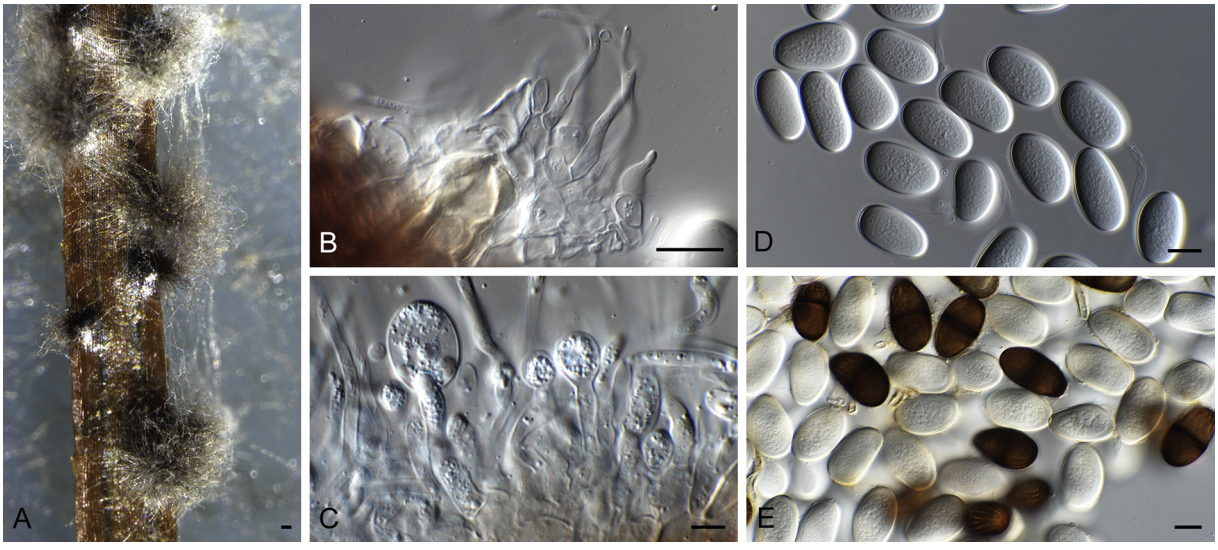


Fig 11 – *Lasiodiplodia vitis* (CBS 124060). (A). Colony sporulating on PNA. (B). Paraphyses. (C). Conidiogenous cells. (D). Young, hyaline conidia. (E). Mature, brown, 1-septate conidia, intermixed with immature conidia. Scale bars: A = 400 μm, all others = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

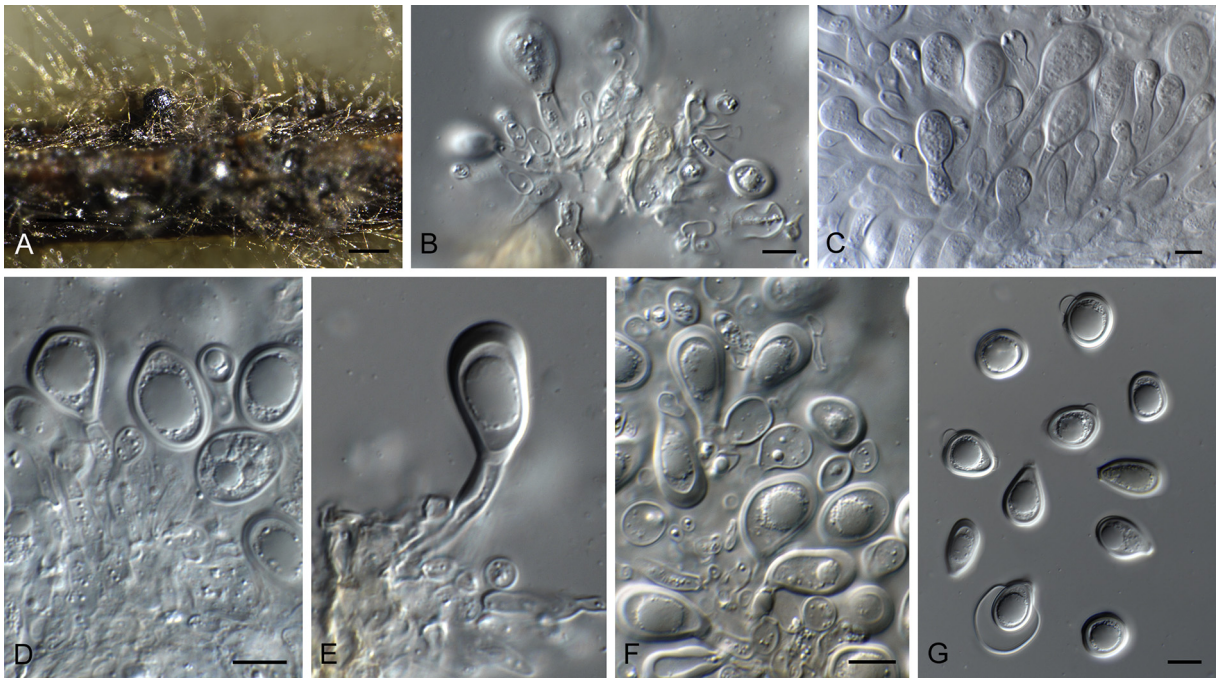


Fig 12 – *Lasiodiplodia sterculiae* (CBS 342.78). (A). Colony sporulating on PNA. (B–F). Conidiogenous cells giving rise to conidia. (G). Mature, thick-walled conidia, some with hyaline appendage. Scale bars: A = 300 μm, all others = 10 μm.

Conidiomata pycnidial, immersed to semi-superficial, separate, globose, dark brown, unilocular, to 300 μm diam; wall of dark brown, thick-walled *textura angularis*, paler and thinner-walled towards the conidiogenous region, often with dark brown superficial hyphae over the surface. *Ostiole* central, single, papillate. *Conidiophores* hyaline, smooth, subcylindrical, 0–2-septate, branched below or not, 8–20 × 2.5–3.5 μm, arising from the inner layers of cells lining the locule. *Conidiogenous cells* hyaline, smooth, subcylindrical to doliiform, 7–12 × 2.5–3.5 μm, discrete, indeterminate, proliferating percurrently with one or two distinct annellations, or proliferating at the same level giving rise to periclinal thickenings. *Conidia* hyaline when young, and not turning brown, even after 2 mo on SNA, thick-walled, obovoid to ellipsoid, straight, broadly rounded at the apex, base truncate, (12–)14–16 (–17) × (8–)10–11 (–12) μm; outer layer frequently forming a swollen, thin-walled appendage-like bulge on 25 % of the conidia observed. *Paraphyses* not observed.

Culture characteristics: Colonies covering dish in 7 d, with sparse aerial mycelium. Colonies on MEA, surface greyish sepia, reverse purplish grey; on PDA surface greyish sepia, reverse pale grey olivaceous; on OA surface grey olivaceous.
Material examined: **Germany**: Braunschweig, on *Sterculia oblonga*, Mar. 1978, S. Bruhn (holotype CBS H-13230, culture ex-type CBS 342.78).
Notes: *Lasiodiplodia sterculiae* was originally deposited in CBS as representative of *Aplosporella sterculiae* (*Sterculia tomentosa*, Erythrea, conidia ellipsoid, chestnut brown, 24 × 9.6 μm; Saccardo & Trotter 1931), but actually represents a species of *Lasiodiplodia* with much smaller conidia. As far as we could establish, this is the first species of *Lasiodiplodia* other than *L. theobromae* described from this host.

Neofusicoccum buxi Crous, *sp. nov.* – MycoBank MB817679; Fig 13.
Etymology: Name refers to the host genus from which it was isolated, *Buxus*.

Conidiomata solitary, immersed, becoming erumpent, brown, up to 250 μm diam, with central ostiole, splitting epidermis with irregular rupture, exuding conidia in a white mucoid mass; wall consisting of 3–5 layers of brown *textura angularis*. *Conidiophores* lining the inner layer of the conidioma, unbranched, subcylindrical, hyaline, smooth, 0–1-septate, 15–30 × 3–8 μm. *Conidiogenous cells* hyaline, integrated, terminal, subcylindrical, proliferating several times percurrently near apex, rarely with minute periclinal thickening, 15–25 × 5–7 μm. *Conidia* hyaline, granular, smooth, thin-walled, subcylindrical, straight to slightly curved, widest in the middle or in the upper third, apex subobtuse, base subtruncate, scar 3–5 μm diam, with minute marginal frill, (25–)30–38 (–45) × (6.5–)7–8 (–9) μm. *Dichomera* synasexual morph not observed.

Material examined: **France**: Provence, near Pont du Gard, on leaf of *Buxus sempervirens*, 24 Oct. 1974, H.A. van der Aa (holotype CBS H-12162; CBS H-12166, culture ex-type CBS 116.75). **Sweden**: Uppsala, Uppsala Botanical Garden, on *Buxus sempervirens*, 12 Nov. 1985, O. Constantinescu, CBS 113714.
Notes: *Dothiorella candollei* causes leaf spots on *Buxus sempervirens* (Batdorf 1995). The two cultures cited above have been deposited in the CBS under the name *Dothiorella candollei*. However, the

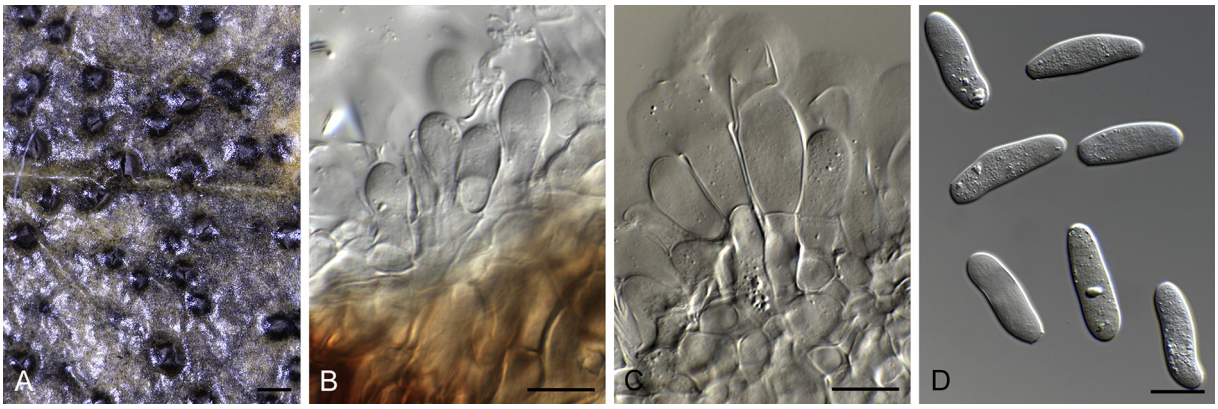


Fig 13 – *Neofusicoccum buxi* (CBS 116.75). (A). Conidiomata *in vivo*. (B, C). Conidiogenous cells. (D). Mature, hyaline, aseptate conidia. Scale bars: A = 250 μm, all others = 10 μm.

basionym, *Sphaeropsis candollei* was never validly published, as it contained reference to the type of *Sphaeria buxi* (Alb. & Schwein.) DC., the epithet that should have been used for this fungus. Since then, the name has become confused with a different fungus occurring on *Buxus*, namely *Hyponectria buxi* (Alb. & Schwein.) Sacc. A new name is thus required for the *Neofusicoccum* species associated with leaf spots of *Buxus*, which is introduced here as *N. buxi*.

Neofusicoccum pistaciae (Zachos *et al.*) Crous, *comb. nov.* – MycoBank MB817680.
Basionym: *Camarosporium pistaciae* Zachos *et al.*, Annls Inst. phytopath. Benaki, N.S. **11**: 57 (1974).
Material examined: **Greece**: near Thessaloniki, on fruit of *Pistacia vera*, 1972, D.G. Zachos (isotype CBS H-499, culture ex-isotype CBS 595.76).
Note: The ex-isotype strain of *Camarosporium pistaciae* clusters in *Neofusicoccum*, and thus a new combination is required for this species.

Neofusicoccum pistaciarum Tao Yang & Crous, *sp. nov.* – MycoBank MB817637; Fig 14.
Etymology: Name refers to the host genus from which it was isolated, *Pistacia*.

Conidiomata solitary, woolly due to abundant external hyphae, erumpent, stromatic, brown, up to 500 μm diam on PNA, with central ostiole, exuding conidia in a white mucoid mass; wall consisting of 3–5 layers of brown *textura angularis*. *Conidiophores* lining the inner layer of the conidioma, subcylindrical, hyaline, smooth, 0–1-septate, 15–30 × 3–5 μm, unbranched or branched below the apical septum. *Conidiogenous cells* hyaline, integrated, terminal, subcylindrical, rarely ampulliform, proliferating several times percurrently near apex, rarely with minute periclinal thickening, 12–20 × 3–4 μm. *Conidia* hyaline, granular, smooth, thin-walled, fusoid-ellipsoidal, straight to slightly curved, widest in the middle or in the upper third, apex subobtuse, base subtruncate, scar 2.5–3 μm diam, with minute marginal frill, (19–) 23–26 (–27) × 5–6 (–6.5) μm. *Dichomera* synasexual morph not observed.

Culture characteristics: Colonies covering dish in 7 d, with cottony aerial mycelium. Colonies on MEA, surface and reverse dirty white; on PDA surface and reverse olivaceous grey; on OA surface grey olivaceous.
Material examined: **USA**: California, Kern County, on *Pistacia vera*, 12 Apr. 2012, T.J. Michailides (holotype CBS H-22722, culture ex-type CBS 113083 = CPC 5263).

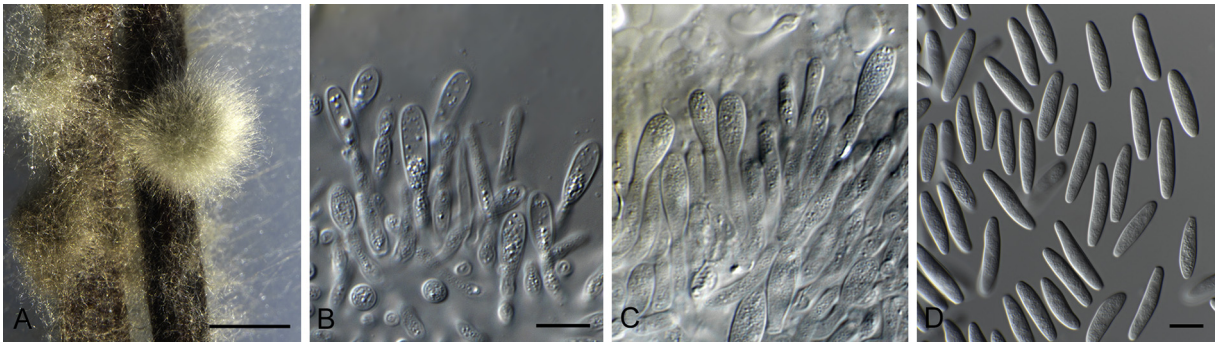


Fig 14 – *Neofusicoccum pistaciarum* (CBS 113083). (A). Colony sporulating on PNA. (B, C). Conidiogenous cells. (D). Mature, Hyaline, aseptate conidia. Scale bars: A = 500 μm, all others = 10 μm.

Notes: Inderbitzin *et al.* (2010) generated a six-gene phylogeny to highlight the extraordinary high species diversity of *Botryosphaeriaceae* on almond in California. In the same paper he also included several isolates from *Pistacia vera*, from which he reported *Botryosphaeria dothidea, Diplodia seriata, Lasiodiplodia theobromae*, *Neofusicoccum mediterraneum*, and *N. parvum*. *Neofusicoccum pistaciarum* represents yet an additional species from this host, which appears distinct from those species presently recognised (Phillips *et al.* 2013).

Neofusicoccum stellenboschiana Tao Yang & Crous, *sp. nov.* – MycoBank MB817638; Fig 15.
Etymology: Name refers to Stellenbosch, the town in South Africa where this fungus was collected.

Conidiomata pycnidial, brown, superficial on PNA, globose, solitary, to 150 μm diam; wall of 3–6 layers of brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, subcylindrical to doliiform, with 1–4 percurrent proliferations near apex, 10–13 × 2–3 μm. *Conidia* hyaline, fusoid, base subtruncate, aseptate, forming two septa before germination, smooth with granular contents, (17–) 19–21 (–22) × (4.5–)5.5–6 μm. *Spermatogonia* and *Dichomera* synasexual morph not observed.

Culture characteristics: Colonies covering dish in 7 d, with cottony aerial mycelium. Colonies on MEA, PDA, and OA surface and reverse dirty white.

Material examined: **South Africa**: Western Cape Province, Stellenbosch, on *Vitis vinifera*, date unknown, F. Halleen (holotype CBS H-22723, culture ex-type CBS 110864 = CPC 4598).
Notes: In the study of *Botryosphaeriaceae* grapevine disease pathogens occurring in South Africa, Van Niekerk *et al.* (2004) treated this isolate as part of the *Neofusicoccum australe* complex, commenting on differences in virulence observed among isolates of this species. With the benefit of additional loci employed in this study, we were now able to show that this isolate was actually closely related to *N. australe*, but represents a distinct species, named here as *N. stellenboschiana*.

Oblongocollomyces Tao Yang & Crous, *gen. nov.* – MycoBank MB817639.

Etymology: Name refers to the elongated conidiomatal necks.

Conidiomata pycnidial, globose to subglobose, covered in short hyphae, superficial, immersed or semi-immersed, developing very long necks, aggregated in clusters; wall of 3–6 layers of brown *textura angularis*. *Paraphyses* intermingled among conidiogenous cells, hyaline, smooth, flexuous, aseptate, rarely branched, with obtuse apices. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to ampuliform, proliferating at the same level to form periclinical thickenings or rarely proliferating percurrently. *Conidia* honey coloured to black-brown, 0–1 (–3)-septate, smooth, hilum truncate, moderately thick-walled, straight to slightly irregularly curved, variable in shape.

Type species: *Oblongocollomyces variabilis* (F.J.J. van der Walt, *et al.*) Tao Yang & Crous.
Notes: *Oblongocollomyces* differs from *Sphaeropsis s.str.* by forming extremely long conidiomatal necks on PNA, or being aggregated in dense clusters on OA, and having conidia that can be up to 3-septate. The long-necked, aggregated conidiomata is a feature that is more common in the family, but atypical for *Sphaeropsis* or *Diplodia*.

Oblongocollomyces variabilis (F.J.J. van der Walt, *et al.*) Tao Yang & Crous, *comb. nov.* – MycoBank MB817640; Fig 16.
Basionym: *Sphaeropsis variabilis* F.J.J. van der Walt, *et al.*, Persoonia 33: 164. 2014.

Conidiomata pycnidial, globose to subglobose, covered in short hyphae, superficial, immersed or semi-immersed, up to 390 μm diam; wall of 3–6 layers of brown *textura angularis*; conidiomata on PNA developing necks up to 1.5 mm long, on OA however, necks absent and conidiomata aggregated in a stroma in clusters of up to 15. *Paraphyses* intermingled among conidiogenous cells, hyaline, smooth, flexuous, up to 60 μm long, 3–4 μm diam, aseptate, rarely branched, with obtuse apices. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, holoblastic, cylindrical to ampuliform, (5.5–)6–11 (–16.5) × 2.5–5.5 (–7.5) μm, proliferating at the same level to form periclinical thickenings or rarely

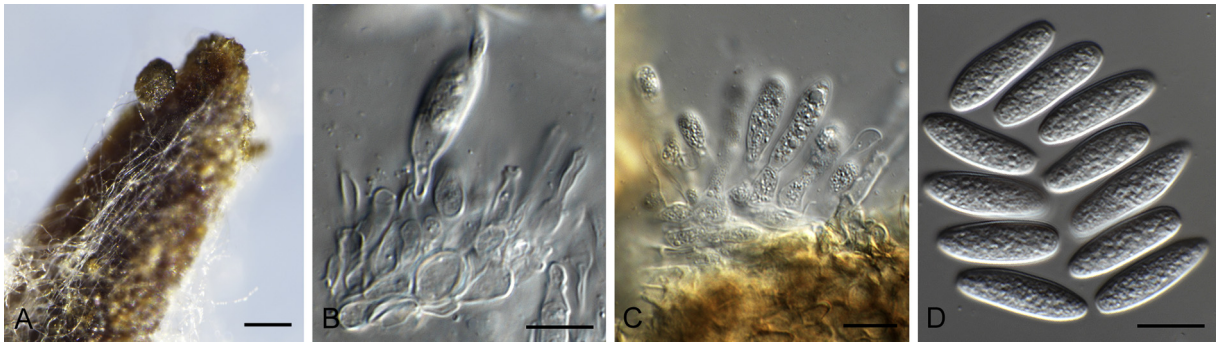


Fig 15 – *Neofusicoccum stellenboschiana* (CBS 110864). (A). Colony sporulating on PNA. (B, C). Conidiogenous cells. (D). Mature, hyaline, aseptate conidia. Scale bars: A = 150 μm, all others = 10 μm.

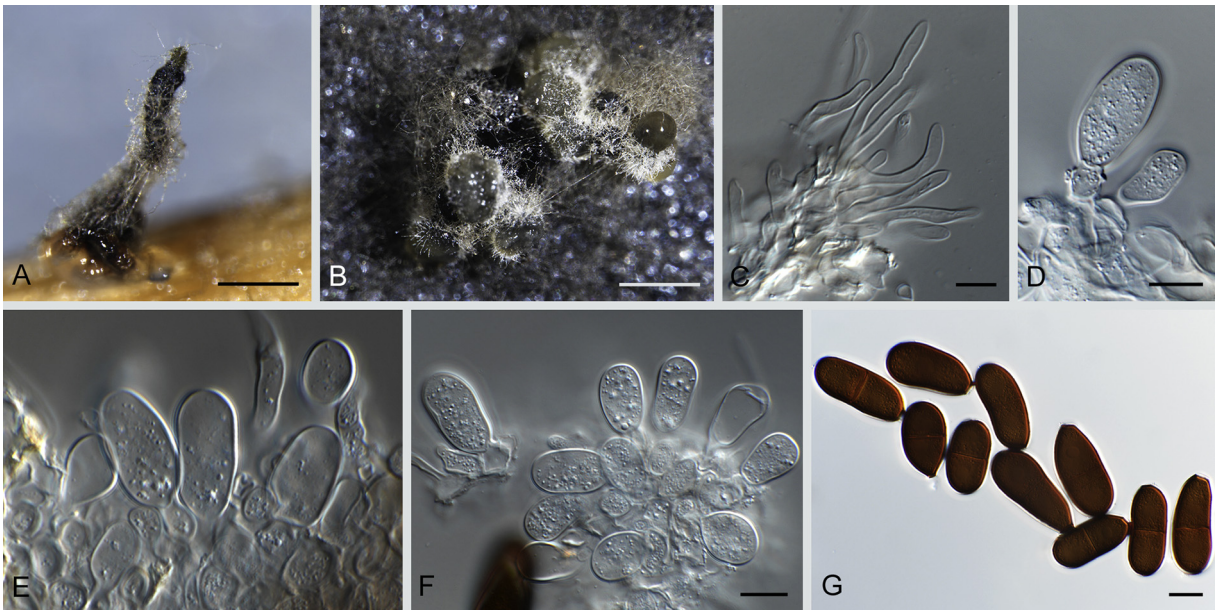


Fig 16 – *Oblongocollomyces variabilis* (CBS 121774). (A). Colony sporulating on PNA, showing long pycnidial neck. (B). Colony sporulating on OA. (C). Paraphyses. (D–F). Conidiogenous cells. (G). Mature, brown, 1-septate conidia. Scale bars: A, B = 400 μm, all others = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proliferating percurrently to form one or two annellations. *Conidia* honey coloured to black-brown, 0–1 (–3)-septate, granular with central guttule, smooth, hilum truncate, 3–4 μm diam, moderately thick-walled, straight to slightly irregularly curved, cylindrical to ovoid or occasionally reniform or allantoid or subclavate, (24–)26.5–33.5 (–37) × (8–)10.5–14 (–17) μm.

Culture characteristics: See Slippers *et al.* (2014).
Material examined: **Namibia**: Windhoek, on *Acacia karroo*, Feb. 2006, F.J.J. van der Walt & J. Roux (holotype PREM 59637, culture ex-type CBS 121774 = CMW 25419).
Notes: With the description of *S. variabilis*, Slippers *et al.* (2014) commented on the fact that this species was allied to *Phaeobotryon*, *Barriopsis*, and *Sphaeropsis*, but eventually chose to allocate it to *Sphaeropsis* based on its typical conidia with obtuse apices and truncate bases. As shown here, however, the reason for this deviating morphology, lies in the fact that it represents a distinct genus.

Endomelanconiopsisaceae Tao Yang & Crous, *fam. nov.* – MycoBank MB817641.

Conidiomata stromatic, immersed, peridermal to subepidermal, separate, irregularly multilocular; walls composed of pale brown, thin-walled *textura angularis*. Dehiscence irregular. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, determinate, discrete, cylindrical, tapered towards the apices, hyaline, smooth, thin-walled. *Conidia* aseptate, pyriform to limoniform, dark brown, thick-walled, smooth, base often protruding and papillate, often with a central guttule and a single germ slit.

Type genus: *Endomelanconiopsis* E.I. Rojas & Samuels.
Notes: *Endomelanconiopsisaceae* resides between *Pseudofusicoccumaceae* and *Melanopsaceae* (Fig 1). The family presently contains a single genus with two species (Phillips *et al.* 2013). The latter two families have no members bearing conidia with germ slits, a feature which appears to be restricted to the *Endomelanconiopsisaceae*.

Pseudofusicoccumaceae Tao Yang & Crous, *fam. nov.* – MycoBank MB817642.

Conidiomata large, immersed to superficial, uni-to multilocular, covered with hyphae; wall of dark brown *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, smooth, cylindrical, proliferating percurrently to form distinct annellations, or proliferating at the same level, giving rise to periclinal thickenings. *Conidia* hyaline, thin-to slightly thick-walled, aseptate, granular, cylindrical, rarely ellipsoid, straight to slightly curved, surrounded by a persistent mucous sheath.

Type genus: *Pseudofusicoccum* Mohali, Slippers & M.J. Wingf.
Notes: Morphologically the family, which is typified by *Pseudofusicoccum*, is similar to *Fusicoccum* asexual morphs of *Botryosphaeria* (*Botryosphaeriaceae*), but distinct in that conidia tend to be more cylindrical in shape, and are encased in a persistent mucoid sheath.

Saccharataceae Slippers, Boissin & Crous, Stud. Mycol. 76: 41. 2013.
Type genus: *Saccharata* Denman & Crous.

Saccharata hawaiiensis Tao Yang & Crous, *sp. nov.* – MycoBank MB817643; Fig 17.

Etymology: Name refers to the Island of Hawaii, where this fungus was collected.

Conidiomata pycnidial, eustromatic, to 450 μm diam, immersed, subepidermal, separate, dark brown, uni-to multilocular, walls consisting of dark brown *textura angularis*, ostiolate. Fusicoccum-like synasexual morph. *Conidiophores* hyaline, smooth, branched, subcylindrical, 1–3-septate, formed from the inner layers of the locule, 20–60 × 3–4.5 μm, intermingled with hyaline, septate paraphyses that are branched, 3–8-septate, up to 90 μm long, 3–4 μm diam. *Conidiogenous cells* discrete or integrated, hyaline, smooth, cylindrical, enteroblastic, proliferating percurrently with numerous apical percurrent annellations, 10–20 × 2.5–3.5 μm. *Conidia* hyaline, thin-walled, aseptate, smooth, fusiform, widest in the middle or upper third of the conidium, with subobtuse apex and a truncate base, (17–)24–30 (–38) × (4–)5–7 (–8) μm. Diplodia-like synasexual morph occurring in same conidiomata as the fusicoccum-like morph. *Conidiophores* hyaline, smooth, branched, cylindrical, 1–3-septate, formed from the inner layers of the conidioma, 15–25 × 2–3 μm. *Conidiogenous cells* phialidic, discrete or integrated, hyaline, smooth, cylindrical, determinate, with prominent periclinal thickening, 6–10 × 2–3 μm. *Conidia* medium brown, thick-walled, finely verruculose, guttulate, aseptate, subcylindrical to narrowly ellipsoid with rounded, but somewhat thickened ends, (8–)10–13 (–15) × 2.5–3.5 μm.

Culture characteristics: Colonies covering dish in 7 d, with sparse to cottony aerial mycelium. Colonies on MEA, PDA, and OA dirty white.
Material examined: **USA**: Hawaii, Manii Floral, on *Protea laurifolia* cv. 'Rose mink', 12 Feb. 1999, P.W. Crous (holotype CBS H-22724, culture ex-type CBS 111787 = CPC 2268).
Notes: The genus *Saccharata* presently contains four species that occur on host plants indigenous to South Africa, such as *Encephalartos* and genera of *Proteaceae* (Crous *et al.* 2008, 2009a), but seem to have also been exported along with these plants to other countries (Crous *et al.* 2013). One further example is *Saccharata hawaiiensis*, which was collected from South African *Protea* cultivated in Hawaii.

Discussion

In this study, a large number of cultures were analysed to produce an expanded phylogenetic backbone for *Botryosphaeriales*, to help delimit novelties at species, genus and family level. The majority of the cultures included here were initially characterised based on their macro-morphology and/or using limited DNA sequence data available at that time. This study stresses the impact DNA sequence data have in providing more accurate identifications of *Botryosphaeriales*, which has consequences for cultivation and trade of plant material (Crous *et al.* 2015b, 2016).

Identification of unknown cultures

One-hundred-and-twenty-six cultures in the CBS culture collection, which were not identified to species level, were re-examined during the course of this study. The largest part (97 cultures), originally listed as '*Botryosphaeria* sp.', were found to represent mainly species of *Diplodia* [24 cultures: *Di. corticola* (1 culture), *Di. pyri* (1 culture), *Di. seriata* (21 cultures) and a *Diplodia* sp. (1 culture)] and *Neofusicoccum* [69 cultures: *N. algeriense* (1 culture), *N. australe* (18 cultures), *N. macroclavatum* (3 cultures), *N. parvum* (13 cultures), *N. pistaciarum* (5 cultures), *N. protearum* (3 cultures), *Neofusicoccum* sp. 1 (3 cultures), *Neofusicoccum* sp. 2 (11 cultures), *Neofusicoccum* sp. 4 (3 cultures), *Neofusicoccum* sp. 6 (1 culture), *Neofusicoccum* sp. 8 (7 cultures), and *N. stellenboschiana* (1 culture)], as well as one culture each of *Lasiodiplodia crassispora*, *Lasiodiplodia mahajangana*, *Macrophomina phaseolina*, and *Saccharata hawaiiensis*. Twelve '*Dothiorella* sp.' cultures were identified as *Do. iranica* (1 culture), *Do. omnivora* (2 cultures), *Do. parva* (2 cultures), *Do. prunicola* (1 culture), *Do. sempervirentis* (2 cultures), as well as *N. luteum* (1 culture), *N. occulatum* (1 culture) and *N. parvum* (2 cultures). Seven *Spencermartinsia* cultures were identified as *Do. citricola* (2 cultures), *Do. mangifericola* (1 culture), *Do. plurivora* (2 cultures), and *Do. striata* (2 cultures). The remaining 10 cultures represent *Aplosporella hesperidica* (was *Aplosporella* sp.), *Bagnisiella* sp. (was *Aplosporella* sp.), *Cophinforma eucalypti* (was *Pseudofusicoccum* sp.), *Diplodia*

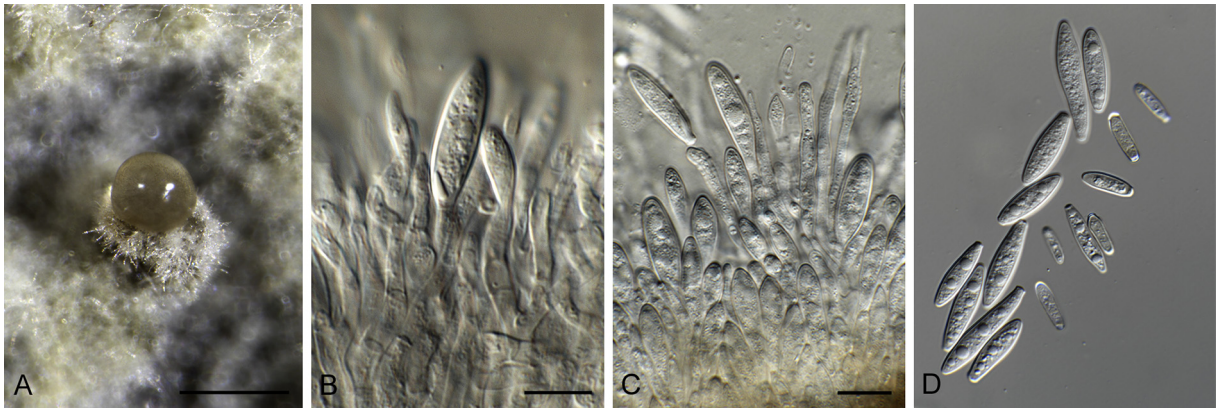


Fig 17 – *Saccharata hawaiiensis* (CBS 111787). (A). Colony sporulating on OA. (B, C). Conidiogenous cells. (D). Mature, hyaline, aseptate conidia, and smaller, pigmented diplodia-like conidia of the synasexual morph. Scale bars: A = 450 μm, all others = 10 μm.

citricarpa (was *Diplodia* sp.), *Diplodia* sp. 1 (was *Diplodia* sp.), *Dothiorella omnivora* (was *Botryodiplodia* sp.), *Dothiorella parva* (was *Otthia* sp.), *Eutiarosporella* sp. (was *Diplodia* sp.), *N. vitifusiforme* or *N. corticosae* (was *Neofusicoccum* sp.), and *Tiarosporella* sp. (was *Tiarosporella* sp.). Several of these previously unidentified species were found to represent taxonomic novelties, namely *Di. citricarpa*, *Di. pyri*, *N. stellenboschiana*, *S. hawaiiensis*, and an additional number of sterile cultures still awaiting further description at species level.

Families of Botryosphaeriales

The *Botryosphaeriales* phylogeny presented here agrees well with general phylogenies in previous studies (Liu *et al.* 2012; Slippers *et al.* 2013; Phillips *et al.* 2013; Wyka & Broders 2016). The topologies of the phylogenetic trees generated from ML, MP, and Bayesian analyses were highly similar and especially the generic and family clades received high support values in all analyses. Our two-gene (LSU and *rpb2*) phylogeny supports the same generic clades as the four-gene and six-gene phylogenies employed by Liu *et al.* (2012) and Slippers *et al.* (2013), respectively. However, some rearrangements in the backbone were observed, such as the position of *Pseudofusicoccum* versus *Endomelanconiopsis* at a more basal position and the position of *Neodeightonia* at the more terminal position. Previous studies have included *Pseudofusicoccum* and *Endomelanconiopsis* as part of the family *Botryosphaeriaceae* [the four-gene, five-gene and six-gene phylogenies of Liu *et al.* (2012), Phillips *et al.* (2013) and Slippers *et al.* (2013), respectively]. This scenario is indicated by 'node 1' (MP-BS/ML-BS/PP: 98/97/1) in our phylogeny (Fig 1); the same node received 55/-/0.95 (MP-BS/ML-BS/PP) support, 100/100 (MP-BS/ML-BS) support, and 89/0.99 (ML-BS/PP) support, in the phylogenies of Liu *et al.* (2012), Phillips *et al.* (2013), and Slippers *et al.* (2013), respectively. A more restrictive scenario would be to exclude the two basal genera, *Pseudofusicoccum* and *Endomelanconiopsis*, from *Botryosphaeriaceae*. This scenario indicated by 'node 2' (MP-BS/ML-BS/PP: 76/98/1 support) in Fig 1; the same node received 73/84/1 (MP-BS/ML-BS/PP) support (*Endomelanconiopsis* was not included in that study), 100/100 (MP-BS/ML-BS) support, and 99/1 (ML-BS/PP) support, in the phylogenies of Liu *et al.* (2012), Phillips *et al.* (2013), and Slippers *et al.* (2013), respectively. An even more conservative scenario would also exclude *Neofusicoccum* and *Dothiorella* from *Botryosphaeriaceae* (Fig 1; scenario indicated by 'node 3', MP-BS/ML-BS/PP: 92/98/1 support); however, this node was only present in the study of Phillips *et al.* (2013) and was poorly supported (MP-BS/ML-BS: -/71). The third scenario would result in a situation where many genera would each receive an own family, which would not be an ideal situation. The second scenario prompted us to describe two novel families to accommodate *Pseudofusicoccum* and *Endomelanconiopsis*. Although these two genera are individually strongly supported, they are consistently placed basal to '*Botryosphaeriaceae*', and their relation to each other and to the other *Botryosphaeriaceae* genera change depending on the type of analysis and the sampling of included loci [also see phylogenies in Liu *et al.* (2012), Phillips *et al.* (2013) and Slippers *et al.* (2013)]. *Pseudofusicoccum* and *Endomelanconiopsis* are also morphologically distinct from other genera in *Botryosphaeriaceae*. In the analysis of Quaedvlieg *et al.* (2013), *Septorioides* was treated as a new monotypic genus typified by *Septorioides pini-thunbergii* in *Botryosphaeriaceae*. However, this genus has since been shown to also cluster in a separate family, namely *Septorioideaceae* (Wyka & Broders 2016).

Genera of Botryosphaeriaceae

Within the *Botryosphaeriaceae*, a combination of SSU-ITS-LSU-*tef1*-*tub2* provided high support for the delimitation of genera (Phillips *et al.* 2013). In the present study, genera could be clearly delimited based on the LSU-*rpb2* dataset. However, *Dothiorella* and *Spencermartinsia* clustered in the same clade intermixing with one another. The two genera were separated based on the presence of apiculi on ascospores of *Spencermartinsia* (Phillips *et al.* 2008, 2013), although this separation was seen as tentative (Slippers *et al.* 2014). Because *Spencermartinsia* rendered *Dothiorella* paraphyletic in our study, we decided to reduce them to a single genus. Furthermore, *Sphaeropsis variabilis* (Slippers *et al.* 2014) was found to cluster separate from *Sphaeropsis*. Morphologically it was found to be distinct, having conidiomata that were either aggregated in dense clusters on OA, or with extremely long necks on PNA, leading us to introduce it as a new genus, *Oblongocollomyces*.

Species complexes

Phillips *et al.* (2013) recommended that at least two loci (ITS-*tef1*) be used for species separation within *Botryosphaeriaceae*. However, the range of variation within a species becomes more apparent as additional isolates are added to the dataset (also see above). In this regard, in addition to ITS-*tef1* sequence data, data from *tub2*, *rpb2* and other loci have at times been found necessary to provide convincing support to separate cryptic species.

In this study, more than 200 *Neofusicoccum* strains were studied. The phylogeny derived from the ITS-*tef1* dataset presented a polytomy in the *N. parvum*/*N. ribis* species complex. Therefore, an ITS-*tef1*-*tub2*-*rpb2* dataset was generated after selecting representatives from each species clade. This phylogeny provided higher support values for each species clade in general, and resolved the polytomy. Sakalidis *et al.* (2011) divided *N. parvum*/*N. ribis* complex into eight species based on the same gene combination.

In another species complex, namely *Diplodia seriata*, several minor sub-clades were detected within the major ITS phylogeny. Furthermore, the phylogenetic relationships among *D. seriata*, *D. pinea*, and *D. scrobiculata* were not well resolved by ITS sequence data (Phillips *et al.* 2007). In subsequent studies *tef1* was combined with ITS to resolve species (Phillips *et al.* 2012, 2013; Alves *et al.* 2014). In the present study, a combination of ITS-*tef1*-*tub2* was used. Compared to the trees generated based on ITS-*tef1*, those from ITS-*tef1*-*tub2* were better supported, and provided a more stable topology.

For *Lasiodiplodia*, the ITS-*tef1*, the loci that have been the most commonly used in literature, revealed (Fig. S1, see TreeBASE) that some recently described species could not be separated as distinct, for example *L. jatrophicola*/*L. iraniensis* (similar to the result of Linaldeddu *et al.* 2015), *L. euphorbicola*/*L. marypalme* (Machado *et al.* 2014; Netto *et al.* 2014), and *L. crassispora*/*L. pyriformis* (Burgess *et al.* 2006; Slippers *et al.*

2014). Unfortunately cultures from those species were not available to us for study and therefore future studies employing additional gene regions would be required to resolve these uncertainties. Inclusion of *tub2* in the concatenated alignment greatly improves the resolution of the resulting tree. Based on only ITS and *tef1*, Sakalidis (2011) identified two putative hybrids in *Lasiodiplodia*. More recently, Cruywagen *et al.* (2017) showed that phylogenies based on five individual genes were incongruent and the authors concluded that several previously described *Lasiodiplodia* species are in fact hybrids. The authors advocate the use of at least four gene loci to judge whether a species could be recognised as distinct. Likewise, Rodríguez-Gálvez *et al.* (2017) identified a putative hybrid between *L. citricola* and *L. parva* during their survey of *Lasiodiplodia* species associated with dieback of mango in Peru.

Gene regions at family and genus level

In the present study a combination of the LSU and *rpb2* gene regions proved to be useful loci to be employed at family level in *Botryosphaeriales*; the combination of these two loci provided phylogenies similar to those of the four-gene, five-gene and six-gene phylogenies of Liu *et al.* (2012), Phillips *et al.* (2013) and Slippers *et al.* (2013), respectively. Furthermore, these gene regions also proved to be highly suited for the delineation of genera within the respective families, leading to the introduction of one new genus. At species level, a combination of at least the ITS and *tef1* gene regions proved adequate for species recognition in many genera, but for genera such as *Diplodia*, *Neofusicoccum*, and *Lasiodiplodia*, additional loci such as *rpb2* and *tub2* are required to establish a robust phylogeny. Based on the derived phylogenies and morphological examinations of the strains investigated, several taxonomic novelties were introduced.

Different combinations of loci have in the past been used in *Botryosphaeriales* to resolve its taxonomy at family level. Schoch *et al.* (2006) employed SSU-LSU-*tef1*-*rpb2*, while Minnis *et al.* (2012) used SSU-ITS-LSU-*rpb1*, Wikee *et al.* (2013) used ITS-LSU-*actA*-*tef1*-*gpdh*, and Slippers *et al.* (2013) used SSU-LSU-ITS-*tef1*-*tub2*-mtSSU. From those studies, a trend was noticeable that with an increasing number of strains and gene markers, more families, genera and species could be resolved in *Botryosphaeriales*. However, LSU proved to be the only gene region that was consistently used in all studies to introduce novel families in the order, and this also proved to be the case in other families recently introduced in *Dothideomycetes* (e.g. Quaedvlieg *et al.* 2013; Crous *et al.* 2015a; Tanaka *et al.* 2015). In contrast, gene regions such as ITS, *tef1* and *tub2* that have several introns, are difficult to align within the order (Crous *et al.* 2015d). These aspects have led to the uncertain taxonomic placement of taxa in several studies. For instance *Pseudofusicoccum* clustered outside the *Botryosphaeriaceae* in the studies of Hyde *et al.* (2013) and Wijayawardene *et al.* (2014), but basal in *Botryosphaeriaceae* in other studies (Slippers *et al.* 2013; Phillips *et al.* 2013). In the present study, an LSU-*rpb2* dataset was generated for phylogenetic comparisons. Since both loci represent slowly evolving genes compared to the intron-rich protein-coding genes often used in previous studies, it proved easier to generate a robust alignment for families within the order. Furthermore, *rpb2* has also been used successfully in different groups of fungi at family level, such as *Teratosphaeriaceae* (Quaedvlieg *et al.* 2013), *Didymellaceae* (Chen *et al.* 2015), *Nectriaceae* (Lombard *et al.* 2015), and at species level, e.g. *Ramularia* (Videira *et al.* 2015), *Alternaria* (Woudenberg *et al.* 2015), *Microdochium* (Hernández-Restrepo *et al.* 2016), *Talaromyces* (Yilmaz *et al.* 2016), and *Chaetomium* (Wang *et al.* 2016). When *rpb2* was employed across the kingdom Fungi, however, results were less encouraging (see Stielow *et al.* 2015), most likely due to a saturation of informative sites which decreases phylogenetic information in that apparent distances largely underestimate the real genetic distances (Philippe *et al.* 2011).

Conclusions

There is an important distinction between 'describing' and 'delimiting' species. DNA phylogeny seeks more to aid in delimiting species – to highlight genetically distinct groups exhibiting levels of sequence divergence suggestive of a new taxon. The combination of molecular support with morphology has generally proven helpful to describe these new taxa (Crous *et al.* 2015a,b,c,d). In *Botryosphaeriales*, taxon circumscription has suffered from insufficient taxon sampling, and too few informative loci. In the present study, the combination of LSU-*rpb2* has proven efficient at family and generic level. In addition, *rpb2* has also proven to work well for species definition, in combination with ITS, *tef1* and *tub2*. With a robust backbone phylogeny for *Botryosphaeriales*, it will in future be easier to delimit novelties at species, genus and family level. This approach again highlights the importance of culture collections as treasure troves of undescribed fungal biodiversity.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2016.11.001.

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