Families and genera of diaporthalean fungi associated with canker and dieback of tree hosts

X.L. Fan¹, J.D.P. Bezerra², C.M. Tian¹, P.W. Crous³,⁴,⁵

INTRODUCTION

Diaporthales represents an important order in Sordariomycetes, containing taxa that are mainly isolated as endophytes, saprobes or plant pathogens on various hosts. The order is characterised by perithecia with elongate beaks, often forming within stromatic tissues, deliquescent paraphyses and asci that generally deliquesce, become detached from the perithecial wall when mature, and have a characteristic refractive apical annulus (Rossman et al. 2007). Members of diaporthalean fungi are responsible for several diseases causing severe damage in plants with economic importance. The most notorious is chestnut blight caused by Cryphonectria parasitica (Cryphonectriaceae) that devastated American chestnut (Castanea dentata) populations in North America (Anagnostakis 1987, Gryzeniaut et al. 2006). Other common diseases include ash anthracnose due to Gnomoniella fraxini and birch canker caused by Cryptosporella platyphylla (Gnomoniaceae) (Redlin & Stack 1988, Fan et al. 2016a), stem-end rot of citrus fruits infected by Diaporthe citri and walnut canker by Diaporthe rostrata (Diaporthaceae) (Huang et al. 2013, 2015, Fan et al. 2015b, Guarinaccia & Crous 2017), willow and walnut canker disease caused by Cytospora chrysosperma (Cytosporaceae) (Fan et al. 2014, 2015a), birch dieback disease resulting from Melanconis stibostoma (Melanconidaceae) (Fan et al. 2016b), walnut dieback disease by Juglandonis juglandina and J. oblonga (in Juglanconidaceae) (Voglmayr et al. 2017), foliar diseases of Eucalyptus by Harknessia spp. (Harknessiaceae) (Crous et al. 2012), and foliar, fruit and stem diseases by Coniella spp. (Schizoparmeaceae) (Alvarez et al. 2016, Marin-Felix et al. 2017).

The classification of Diaporthales has changed drastically over the past decades because of the plasticity and variability in morphology. The order Diaporthales and Valsaerae were first introduced by Nannfeldt (1932), based on subfamilies Eu-Diaportheen and Valseae in Diaporthaceae proposed by Von Höhnel (1917). Later, Diaporthales and ‘Valsaerae’ (now Cytosporaceae, and referred to as such below) were recognised in the Diaporthales by Von Arx & Müller (1954). Kobayashi (1970) proposed Diaporthaceae (including Valsa = Cytospora) in a wide concept including all taxa accepted in Diaporthales by Barr (1978). Wehmeyer & Hanlin (1975) accepted three families within this order, including non-allantoid sporulated Gnomoniaceae and Diaporthaceae separated on the presence or absence of a stroma, and Cytosporaceae with allantoid ascospores. Barr (1978) arranged four families (Gnomoniaceae, Melanconidaceae, Pseudomelanconidaceae and Cytosporaceae) in Diaporthales based on basal position of ascomata and thin or firm ascospore walls without special emphasis on allantoid or non-allantoid ascospores. Families within the Diaporthales have been segregated by several mycologists to utilise various criteria: stromatic tissues, arrangement of ascomata in the stroma or substrate, and ascospore shape, e.g., four families (Cytosporaceae, Endoxylaceae, Gnomoniaceae and Melanconidaceae) by Monod (1983), three families (Cytosporaceae, Melanconidaceae and Phyllachoraceae) by Cannon (1988), while Hawksworth et al. (1995) merged the Cytosporaceae, Gnomoniaceae and Melanconidaceae proposed by Barr (1990) to Cytosporaceae and Melanconidaceae. These changes and confusions suggested that phenotypic characters alone were unable to provide sufficient evidence to resolve phylogenetic and evolutionary patterns among these fungi.

Key words

Ascomycota
Phylogeny
Sordariomycetes
Taxonomy

Abstract

In this study we accept 25 families in Diaporthales based on phylogenetic analyses using partial ITS, LSU, rpb2 and tef1-α gene sequences. Four different families associated with canker and dieback of tree hosts are morphologically treated and phylogenetically compared. These include three new families (Diaporthostomataceae, Pseudomelanconidaceae, Synnemasporellaceae), and one new genus, Dendrostoma (Erythrogloeaceae). Dendrostoma is newly described from Malus spectabilis, Osmanthus fragrans and Quercus acutissima having fusoid to cylindrical, bicellular ascospores, with three new species namely D. macil, D. osmanthi and D. quercinum. Diaporthostomataceae is characterised by conical and discrete perithelia with bicellular, fusoid ascospores on branches of Machilus leptophylla. Pseudomelanconidaceae is defined by conidiogenous cells with apical collarettes and discrete annellations, and the inconspicuous hyaline conidial sheath when mature on Caryya cathayensis, compared to morphologically similar families Melanconidaceae and Juglanconidaceae. Synnemasporellaceae is proposed to accommodate fungi with synnematosum conidiolema, with descriptions of S. toxicodendri on Toxicodendron sylvestre and S. aculeians on Rhus copallina.

Article info

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Molecular studies on fungi began in the early 1990s, and since then ribosomal DNA sequence data were accepted as the standard gene loci for fungi (Berbee & Taylor 1992, Schoch et al. 2012). Zhang & Blackwell (2001) recognised three lineages in *Diaporthales*, while Castlesbury et al. (2002) postulated six major lineages, namely the *Cryphonectria-Entothia* complex, *Cytosporaceae* s.s., *Diaporthaceae* s.s., *Gnomoniaceae* s.s., *Melanconidiaeae* s.s. and the *Schizoporemae* complex. When Rossman et al. (2007) reviewed the *Diaporthales*, nine families were recognised, i.e., *Cryphonectriaceae*, *Cytosporaceae*, *Diaporthaceae*, *Gnomoniaceae*, *Melanconidiaeae*, *Pseudovalsaceae*, *Schizoporemae* and *Togniaceae*. Kirk et al. (2008) added *Melanoma* s.s. and listed 10 families in this order, whereas Jaklitsch & Voglmayr (2012) placed *Melanoma* s.s. within *Xylariales* rather than *Diaporthales*. Subsequently, the *Pseudoplagiostomataceae*, *Harknessiaceae*, *Macrohiilarisaeae* and *Tirisporellaceae* were also added to the *Diaporthales* (Cheewangkoon et al. 2010, Crous et al. 2012, 2015, Suetrong et al. 2015). Voglmayr & Jaklitsch (2014) resurrected *Stibosporaceae*, while the *Togniaceae* and *Tirisporellaceae* were reallocated to the *Togniniales* and *Tirisporellales* (Gramaje et al. 2015, Jones et al. 2015). Later, *Lamproconidiaeae* and *Juglanconidiaeae* were proposed as new families in this order (Norphanphou et al. 2016, Voglmayr et al. 2017). The recent outline of *Diaporthales* published by Senanayake et al. (2017) used morphological and phylogenetic evidence to introduce seven new families and accepted a total of 21 families in the order. In spite of these changes, the phylogenetic placement of many genera in the *Diaporthales* remains unknown, and many families still wait to be elucidated.

During the trips to collect forest pathosigns that cause canker or dieback diseases in China, several diaporthalean taxa associated with various disease symptoms were collected in Jiangxi and Zhejiang Provinces, China. Because the higher-level phylogeny of many genera within the *Diaporthales* remains largely unresolved, this project was initiated to address this issue. In this paper, we propose three new families and one new genus as well as several new species.

**MATERIALS AND METHODS**

**Isolation**

Fresh specimens of diaporthalean fungi were collected from infected branches of seven hosts during collection trips in China (Table 1). A total of 20 isolates were established by removing a mucoid spore mass from ascomata or conidiomata, spreading the suspension on the surface of 1.8 % potato dextrose agar (PDA), and incubating at 25 °C for up to 24 h. Single germination tests were performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v. 3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

**Morphology**

Species identification was based on morphological features of the ascomata or conidiomata produced on infected plant tissues and micromorphology, supplemented by cultural characteristics. Cross-sections were prepared by hand using a double-edge blade under a dissecting microscope. At least 10 conidiomata/ascomata, 10 asci and 30 conidia/ascospores were measured to calculate the mean size and standard deviation (SD). Microscopic photographs were captured with a Nikon digital sight DS-Ri2 high definition colour camera, using differential interference contrast (DIC) illumination and the Nikon software NIS-Elements D Package v. 3.00. Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for the manual editing. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004). Colony diameters were measured, and the colony colours described after 3 wk according to the colour charts of Rayner (1970).

**DNA extraction, amplification and sequencing**

Genomic DNA was extracted using a modified CTAB method, with fungal mycelium harvested from PDA plates with collphane (Doyle & Doyle 1990). The ITS region was amplified with the primers ITS1 and ITS4 (White et al. 1990), the LSU region with the primers LROR and LR5 (Vilgalys & Hester 1990), the rpb2 region with primers RRPB2-5F and RRPB2-7C-R (Liu et al. 1999), and the *tef1*-a gene with the primers EF1-728F and EF1-986R (Carbone & Kohlh 1999). The PCR mixture for all regions consisted of 1 μL genomic DNA, 3 mM MgCl₂, 20 μM of each dNTP, 0.2 μM of each primer and 0.25 U BIOTAQ DNA polymerase (Biotime). Conditions for PCR of ITS and LSU genes constituted an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s at 51 °C and 1 min at 72 °C, and a final denaturation step of 8 min at 72 °C. For the *tef1*-a gene was performed using an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s at 56 °C and 1 min at 72 °C, and a final denaturation step of 8 min at 72 °C. For the *rpb2* amplification, conditions consisted of five cycles of 45 s at 95 °C, 45 s at 56 °C and 2 min at 72 °C, then five cycles with a 53 °C annealing temperature and 30 cycles with a 50 °C annealing temperature. The DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v. 3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

**Molecular data analyses**

DNA sequences generated by each primer combination were used to obtain consensus sequences using SeqMan v. 7.1.0 in the DNASTAR Lasergene Core Suite software (DNASTAR Inc., Madison, WI, USA). Reference sequences were selected based on ex-type or ex-epitype sequences available from relevant published literature (Rossman et al. 2007, Cheewangkoon et al. 2010, Crous et al. 2012, 2015, Suetrong et al. 2015, Norphanphou et al. 2016, Hongsan et al. 2017, Senanayake et al. 2017, Voglmayr et al. 2017) (Table 1). All sequences were aligned using Mafft v. 6 (Katoh & Toh 2010) and edited manually using MEGA v. 6 (Tamura et al. 2013). Phylogenetic analyses were performed using PAUP v. 4.0b10 for maximum parsimony (MP) analysis (Swofford 2003), MrBayes v. 3.1.2 for Bayesian Inference (BI) analysis (Ronquist & Huelsenbeck 2003), and PhyML v. 7.2.8 for Maximum Likelihood (ML) analysis (Guindon et al. 2010). The first analyses were performed on the combined multi-genome dataset (ITS, LSU, *rpb2, tef1*-a) to compare isolates of *Diaporthales* species to ex-type sequence data from recent studies (Table 1).

A partition homogeneity test (PHT) with heuristic search and 1000 replicates was performed using PAUP v. 4.0b10 to test the discrepancy among the ITS, LSU, *rpb2* and *tef1*-a sequence datasets in reconstructing phylogenetic trees. Maximum parsimony (MP) analysis was run using a heuristic search option of 1000 search replicates with random-additions of sequences with a tree bisection and reconnection (TBR) algorithm. MxTrees were set to 5000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). Maximum
| Species | Culture | Location | Host | GenBank accession numbers |
|---------|---------|----------|------|--------------------------|
| Apiosporopsis carnea | CBS 771.79 | Switzerland | Carpinus betulus | GU797406 JF838338 NA NA |
| Apiosporopsis sp. | Masuya 11AZ-1 | Japan | Alnus firma | KX430118 KX430074 NA NA |
| Aphonknessia insueta | CBS 11377 | Brazil | Eucalyptus peltata | KX430112 KX430070 NA NA |
| Asterosporium asterospermum | MFLUCC 15-3555 | Italy | Fagus sylvatica | JF681947 EU863088 NA |
| Austrocarpospora | CBS 152240 | China | Betula platyphylla | NA NA NA |
| A. koreana | MFLUCC 17-1669 | South Africa | Hibiscus sp. | NA NA NA |
| A. musaeicarpa var. habiscoiae | AR 3534 + CBS 109757 | Austria | Quercus robur | NA NA NA |
| A. umbranatum | MFLUCC 15-1110 | Italy | Quercus sp. | MFL604710 MFL776110 NA |
| Cryptosporis macrospora | AR 344 + CBS 109764 | Russia | Quercus mongolica | MFL604710 MFL776110 NA |
| Cryptosporis nitzschiiae | AR 3352 + CBS 109772 | Austria | Juniperus communis | MFL604710 MFL776110 NA |
| Cryptosporis parastica | ATCC 38755 | USA | Castanea dentata | MFL604710 MFL776110 NA |
| Cryptodiaporthe ascella | CBS 109765 + AFTOL-ID 1238 | Austria | Aesculus hippocastanum | DQ325350 AF084342 EU199138 GU354004 |
| Cryptospora betulae | AR 3469 + CBS 109763 | Austria | Betula pendula | EU199138 EU199139 EU199140 |
| Cryptospora hypodermia | AR 3552 | Austria | Ulmus minor | EU199138 EU199139 EU199140 |
| Cryptospora pubescens | AR 3496 + CBS 109765 | Austria | Alnus incana | EU199138 EU199139 EU199140 |
| Cytospora chinensis | AR 3345 + CBS 109766 | China | Ulmus rubra | MFL604710 MFL776110 NA |
| Cytospora chinensis | AR 3512 | China | Ulmus rubra | MFL604710 MFL776110 NA |
| Cytospora sp. | AR 3512 | China | Ulmus rubra | MFL604710 MFL776110 NA |
| Cytospora sp. | AR 3416 + CBS 109756 | Russia | Quercus mongolica | MFL604710 MFL776110 NA |
| Dendrostoma malaisiae | CFCC 52012* | China | Malus spectabilis | MFL604710 MFL776110 NA |
| Dendrostoma osmanthi | CFCC 52012* | China | Osmantus fragrans | MFL604710 MFL776110 NA |
| Dendrostoma parvum | CFCC 52012* | China | Osmantus fragrans | MFL604710 MFL776110 NA |
| Dendrostoma quercinum | CFCC 52012* | China | Quercus species | MFL604710 MFL776110 NA |
| Diaporthe decedens | AR 3435 + CBS 109772 | Austria | Corylus avellana | MFL604710 MFL776110 NA |
| Diaporthe detrusa | AR 3424 + CBS 109770 | Austria | Berberis vulgaris | MFL604710 MFL776110 NA |
| Diaporthe eres | AR 3538 + CBS 109767 | Austria | Acer campestre | MFL604710 MFL776110 NA |
| Diaporthe corylina | CBS 121124 | China | Corylus avellana | MFL604710 MFL776110 NA |
| Diaporthella sp. | CN 5 | China | Corylus avellana | MFL604710 MFL776110 NA |
| Diaphorosphella cedricola | CFCC 51989 | China | Cercis chinensis | MFL604710 MFL776110 NA |
| Diaphorosphella cedricola | CFCC 51989 | China | Cercis chinensis | MFL604710 MFL776110 NA |
| Diaphorosphella cedricola | CFCC 51999 | China | Cercis chinensis | MFL604710 MFL776110 NA |
| Diaphorosphella cedricola | CFCC 51999 | China | Cercis chinensis | MFL604710 MFL776110 NA |
| Diculoides eucalypti | CPC 17650 | Australia | Eucalyptus viminalis | MFL604710 MFL776110 NA |
| Diculoides eucalyptorum | CBS 132184 + CPC 17648 | Australia | Eucalyptus globulus | MFL604710 MFL776110 NA |
| Ditylota diplopha | AR 3423 + CBS 109748 | Brazil | Hymenaea courbarillii | MFL604710 MFL776110 NA |
| Dryosporium caesarea | CBS 130776 | Australia | Bankia saxata | MFL604710 MFL776110 NA |
| Dryosporium caesarea | CPC 13043 | Australia | Eucalyptus sp. | MFL604710 MFL776110 NA |
| Dryosporium caesarea | CPC 13043 | Australia | Eucalyptus sp. | MFL604710 MFL776110 NA |
Molecular phylogenetic analyses

The alignment based on the sequence dataset (ITS, LSU, rpb2 and tef1-α) included 122 ingroup taxa, comprising 3261 characters in the aligned matrix. Of these, 1562 characters were constant, 184 variable characters were parsimony-uninformative and 1515 characters were parsimony informative. The MP analysis resulted in 119 equally most parsimonious trees (TL = 8082, CI = 0.385, RI = 0.761, RC = 0.293) and the first tree is shown in Fig. 1. For BI analyses, the general time reversible model, additionally assuming a proportion of invariant sites with gamma-distributed substitution rates of the remaining sites, was used.

RESULTS

likelihood (ML) analysis was performed with a GTR site substitution model, including a gamma-distributed rate heterogeneity and a proportion of invariant sites (Guindon et al. 2010). The branch support was evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). MrModeltest v. 2.3 was used to estimate the best nucleotide substitution model settings for each gene (Posada & Crandall 1998). Bayesian inference (BI) was performed based on the DNA dataset from the results of the MrModeltest, using a Markov Chain Monte Carlo (MCMC) algorithm in MrBays [3.1.2] (Ronquist & Huelsenbeck 2003). Two MCMC chains were run from random trees for 100 M generations and stopped when average standard deviation of split frequencies fell below 0.01. Trees were saved every 1000 generations. The first 25% of trees were discarded as the burn-in phase of each analysis, and the posterior probabilities (BPP) were calculated from the remaining trees (Rannala & Yang 1996). Phylograms were shown using FigTree v. 1.3.1 (Rambaut & Drummond 2010). Novel sequences generated in the current study were deposited in GenBank (Table 1) and the aligned matrices used for phylogenetic analyses in TreeBASE (www.treebase.org; accession number: S22175).

Table 1 (cont.)

| Species | Culture | Location | Host | GenBank accession numbers |
|---------|---------|----------|------|--------------------------|
| Melanocniella ellipta | BPI 878343 | USA | Carpinus caroliniana | JQ026721 JQ026727 JQ026339 JQ026406 |
| Melanocniella hyperbota | AR 3832 + CBS 131492 | Austria | Carpinus betulus | JQ026278 JQ026278 NA NA |
| Melanocniella spodiaea | MSH | Austria | Carpinus betulus | JQ026298 JQ026298 JQ026364 JQ026431 |
| Melanocniella alni | AR 3748 | Austria | Alnus viridis | EU199195 EU199130 EU199153 NA |
| Melanocniella betulae | CFCC 5047 | China | Betula albosinensis | KT732952 KT732971 KT732984 KT733001 |
| Melanocniella fiterana | CFCC 5047 | China | Betula albosinensis | KT732955 KT732974 KT732987 KT733001 |
| Melanocniella marginalis | AR 342 + CBS 109744 | Canada | Alnus rubra | EU199197 AF408373 EU219301 EU222191 |
| Melanocniella stiltostoma | CFCC 5047 | China | Betula platyphylla | KT732956 KT732975 KT732988 KT733005 |
| Nakatea oryzae | CBS 243.76 | NA | NA | KM484861 DQ341498 NA NA |
| Ophiodiaphorthe cyathae | YMJ1364 | China | Cyathae lepifera | JX570889 JX570891 JX570893 NA |
| Pachytype prionces | Rogers 5 | USA | NA | FJ533282 NA NA |
| Pachytype rimosa | FF1066 | Costa Rica | NA | NA FJ533281 NA NA |
| Paradiaphorthe artemisiae | MFLUCC 14-0850 | Italy | Artemisia sp. | MF190155 MF190100 NA NA |
| Phaeoappendispora thailandensis | MFLUCC 17-1663 | Italy | Artemisia sp. | MF190156 MF190101 NA NA |
| Phaeoappendispora appendiculata | CBS 12809 + D76 | Austria | Acer campestre | KF570156 KF570156 NA NA |
| Phragmoporia conformis | AR 3632 + CBS 109783 | Canada | Alnus rubra | DQ332567 AF408377 NA NA |
| Pilagiostra euruphoaiae | CBS 340.78 | Netherlands | Euphorbia palustris | EU199198 AF408382 DQ386664 NA |
| Pilagiostra salticium | AR 3453 + CBS 109775 | Austria | Salix sp. | DQ332567 AF408345 EU199141 EU222191 |
| Prosopidica mexicana | CBS 113530 | USA | Prosopis glandulosa | AT720710 NA NA NA |
| Pseudomelanconis caryae | CFCC 52110* | China | Carya cathayensis | MG682082 MG682024 MG682042 MG682062 |
| Pseudomelanconis caryae | CFCC 52111* | China | Carya cathayensis | MG682083 MG682023 MG682043 MG682063 |
| Pseudomelanconis caryae | CFCC 52112* | China | Carya cathayensis | MG682084 MG682024 MG682044 MG682064 |
| Pseudomelanconis caryae | CFCC 52113* | China | Carya cathayensis | MG682085 MG682025 MG682045 MG682065 |
| Pseudoplagoistroc eucaelyphi | CBS 124807 | Venezuela | Eucalyptus urophylla | GU973512 GU973506 NA NA |
| Pseudoplagoistroc eucaelyphi | CBS 116382 | Thailand | Eucalyptus camaldulensis | GU973514 GU973408 NA NA |
| Pseudoplagoistroc eucaelyphi | CBS 115722 | Australia | Eucalyptus camaldulensis | GU973535 GU973410 NA NA |
| Pseudopiocacida mexicana | CBS 113530 | USA | Prosopis glandulosa | AT720710 NA NA NA |
| Pycyricia grisea | Ina160 | Japan | NA | AB026819 AB026819 NA NA |
| Rossmania ukurunduensis | AR 348 | Russia | Acer ukurunduensis | NA EU683375 NA NA |
| Sillia ferruginea | AR 3440 + CBS 126567 | Austria | Corylus avellana | JF681959 EU683376 NA NA |
| Stegosporium protophyllum | CBS 117041 | Austria | Acer pseudoplatanus | NR126119 EU039992 NA NA |
| Stegosporium sect. pseudophyllae | CBS 124487 | UK | Acer hildreichi | KF570105 KF570156 KF570190 NA |
| Stobispora macroaspera | CBS 121883 | Austria | Carpinus betulus | JX517290 JX517299 KF570196 NA |
| Sydwoiella depressula | CBS 813.79 | Switzerland | Rubus sp. | NA EU683077 NA NA |
| Sydwoiella fenestras | AR 3777 + CBS 125530 | Russia | Chamerion angustifolium | JF681956 EU683078 NA NA |
| Syneumasporella aurea | CFCC 52095* | China | Rhus chinensis | MG682087 MG682025 MG682045 MG682066 |
| Syneumasporella aurea | CFCC 52093* | China | Rhus chinensis | MG682087 MG682025 MG682045 MG682067 |
| Syneumasporella aurea | AR 3878 + CBS 126566 | USA | Rhus glabra | NA EU255134 NA NA |
| Syneumasporella toxicoidendri | CFCC 52097 | China | Toxicodendron sylvestre | MG682089 MG682025 MG682045 MG682068 |
| Syneumasporella toxicoidendri | CFCC 52095* | China | Toxicodendron sylvestre | MG682087 MG682025 MG682045 MG682067 |
| Syneumasporella toxicoidendri | CFCC 52096* | China | Toxicodendron sylvestre | MG682087 MG682025 MG682045 MG682067 |
| Syneumasporella toxicoidendri | CFCC 52099 | China | Toxicodendron sylvestre | MG682089 MG682025 MG682045 MG682068 |
Fig. 1 Phylogram of Diaporthales resulting from MP analysis based on combined ITS, LSU, rpb2 and tef1-α. MP and ML bootstrap support values above 70% are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Type species are in bold. Strains obtained in the current study are in blue. — Scale bar = 200 changes.
model for the rpb2 locus was the Tamura-Nei model, additionally assuming a proportion of invariant sites with gamma-distributed substitution rates of the remaining sites (TNN + I + G). The MP and ML bootstrap support values above 70% are shown at the first and second position, respectively. Branches with significant Bayesian posterior probability (≥ 0.95) in Bayesian analyses were thickened in the phylogenetic tree. The phylogram based on four genes indicated 24 known lineages, representing 22 known families and two unknown taxa lacking typification studies, namely Diaporthella and Phaeoappendispora. Four new lineages belonging to the Diaporthales, distinct from all known taxa, are herein described as three new families and a new genus in Erythroleaceae (Fig. 1).

Taxonomy

**Diaporthostomataceae** X.L. Fan & C.M. Tian, fam. nov. — MycoBank MB823983

*Diaporthostoma* X.L. Fan & C.M. Tian, gen. nov. — MycoBank MB823984

Etyymology. Name derived from the morphological similarity with the genus *Diaportha*.

Type species. *Diaporthostoma machili* X.L. Fan & C.M. Tian.

Sexual morph: Pseudostromata immersed in host bark, slightly erumpent from the bark surface. Ectostromatic disc yellowish to dark grey, nearly flat, ovoid to ellipsoid. Central column beneath the disc more or less conical. Stromatic zones lacking. Perithecia conical, surrounding the ectostromatic disc. Ostioles single, dark grey to black. Paraphyses deliquescent. Asci 8-spored, with an apical ring. Ascospores hyaline, fusoid, bicellular. Asexual morph: not observed.

Notes — The current phylogenetic analyses placed the new family *Diaporthostomataceae* in a highly supported clade (MP/ML/BI = 100/100/1) closely related to *Diaporthosporaceae*. *Diaporthostomataceae* is morphologically distinct from *Diaporthosporaceae* (Yang et al. 2018) by discrete perithecia and fusoid, straight to curved ascospores with a median septum. 

**Diaporthostoma** X.L. Fan & C.M. Tian, gen. nov. — MycoBank MB823984

Etyymology. Name derived from the morphological similarity with the genus *Diaportha*.

Type species. *Diaporthostoma machili* X.L. Fan & C.M. Tian.

Sexual morph: Pseudostromata immersed in host bark, slightly erumpent from the bark surface. Ectostromatic disc yellowish to dark grey, nearly flat, ovoid to ellipsoid. Central column beneath the disc more or less conical. Stromatic zones lacking. Perithecia conical, surrounding the ectostromatic disc, regularly scattered. Ostioles single, dark grey to black. Paraphyses deliquescent. Asci oblong to cylindrical-clavate, 8-spored,
2–3-seriate, with a more or less distinct apical ring. *Ascospores* hyaline, smooth, fusoid, multiguttulate, straight to curved, bicellular, with an inconspicuous median septum. **Asexual morph:** not observed.

**Diaporthostoma machili** X.L. Fan & C.M. Tian, sp. nov. — MycoBank MB823985; Fig. 2

*Etymology:* Name derived from the host genus, *Machilus.*

*Sexual morph: Pseudostromata* immersed in host bark, slightly erumpent, 400–700 µm diam. **Ectostromatic disc** yellowish to dark grey, nearly flat, ovoid to ellipsoid, 120–140 µm diam. **Central column** beneath the disc more or less conical. *Stromatic zones* lacking. *Perithecia* conical, surrounding the ectostromatic disc, regularly scattered, 380–420 µm diam. **Ostioles** single, dark grey to black, 65–85 µm diam. **Paraphyses** deliquescent. **Ascii** 8-spored, with an apical ring, groupded at the base with other asci, becoming detached from the perithecial wall. **Ascospores** hyaline, fusoid to cylindric-bicellular. **Asexual morph:** folliculous, associated with leaf spots. *Conidiomata* epiphyllous, subepidermal, sometimes eumycetous, acervular or subglobose, brown to black or yellow-orange, amphigenous, opening by irregular rupture, wall of 2–6 layers of orange-brown *textura angularis*, exuding slimy orange masses of conidia. *Conidiophores* hyaline to olivaceous, smooth, guttulate or not, thin-walled, ellipsoid, fusoid, ovoid to somewhat obclavate, straight to curved, apex subobtuse, obtusely rounded, base truncate, with prominent marginal frill, or dimorphic, intermixed in same conidiomata. *Macroconidia* broadly ellipsoid to obvoid, hyaline, smooth, granular to guttulate, thick-walled, apex obtuse, base flattened. *Macroconidia* hyaline, smooth, guttulate, fusoid-ellipsoid, acutely rounded at apex, truncate at base (emended from Senanayake et al. 2017).

*Notes* — The family *Erythrogloeaceae* was recently introduced by Senanayake et al. (2017) to accommodate *Chryscrypta, Disculoides* and *Erythrogloeum* species having epiphylous acervuli, and subcylinndrical to ampulliform conidiogenous cells. These authors did not report any sexual morph associated with these genera. During our investigation, phylogenetic inferences using DNA sequences from some materials with a sexual morph placed these samples in a highly supported clade (MP/ML/BI = 100/100/1) in the *Erythrogloeaceae*. The family *Erythrogloeaceae* is emended here to include the morphological

![Fig. 2](image-url)
features of the new sexual morphs observed during our study. These fungi have typical diaporthalean perithecia with clavate asci, and fusoid to cylindrical, bicellular ascospores.

**Dendrostoma** X.L. Fan & C.M. Tian, gen. nov. — MycoBank MB823986

**Etymology.** Name derived from pseudostromata emerging from woody host tissue.

**Type species.** *Dendrostoma mali* X.L. Fan & C.M. Tian.

**Sexual morph:** Pseudostromata small to large, distinct, circu-
lar, erumpent, consisting of an inconspicuous, usually orange ectostromatic disc, semi-immersed to superficial, causing a pustulate bark surface. **Ectostromatic disc** flat or concave, orange, surrounded by bark flaps. **Central column** beneath the disc more or less conical. **Stromatic zones** lacking. **Perithecia** conspicuous, umber to fuscous black, embedded in orange to umber pseudostromatic tissue, regularly scattered, surrounding the ectostromatic disc, with small to long ostioles that emerge within the ectostromatic disc. **Ostioles** flat in the disc or sometimes slightly projecting, cylindrical, sometimes obscuring the disc, covered by an orange, umber to fuscous black crust. **Paraphyses** deliquescent. **Asci** fusoid, 8-spored, 2–3-seriate, with an apical ring, becoming detached from the perithecial wall. **Ascospores** hyaline, fusoid to cylindrical, symmetrical to asymmetrical, straight to curved, biseriate, with a median septum, constricted at the septum, smooth, multiguttulate. **Asexual morph:** observed on PDA. **Conidiomata** pycnidial, hemispheric, somewhat erumpent, coated with aerial mycelium. **Conidiophores** hyaline to amber. **Conidiogenous cells** enteroblastic, polyphialidic, hyaline, verruculose, ampulliform to doliiform. **Conidia** hyaline, aseptate, ovoid to ellipsoid, or fusoid.

Notes — The current phylogenetic analyses placed the new genus *Dendrostoma* in a highly supported clade (MP/ML/ BI = 100/100/1) closely related to other genera in *Erythroglaceae* (Senanayake et al. 2017). *Dendrostoma* is described based on the typical diaporthalean perithecia with clavate asci and fusoid to cylindrical, bicellular ascospores. This study describes one genus and four species from China, and the host association appears to provide an important character for reliable identification. However, further collections are needed to confirm the host ranges and geographical distributions.

**Dendrostoma mali** X.L. Fan & C.M. Tian, sp. nov. — MycoBank MB823987; Fig. 3

**Etymology.** Name derived from the host genus, *Malus*.

**Pseudostromata** erumpent, consisting of an inconspicuous orange ectostromatic disc, semi-immersed to superficial, causing a pustulate bark surface, 1300–2100 µm diam. **Ectostromatic disc** flat or concave, orange, or brown to black, sometimes concealed by ostioles, surrounded by bark flaps, 350–800 µm diam. **Central column** yellowish to brownish. **Stromatic zones** lacking. **Perithecia** conspicuous, umber to fuscous black, regularly scattered, surrounding the ectostromatic disc, 300–500 µm diam. **Ostioles** 2–6 per disc, flat in the disc or sometimes slightly projecting, cylindrical, covered by an orange, umber to fuscous black crust, 70–100 µm diam. **Paraphyses** deliquescent. **Asci** fusoid, 8-spored, biseriate, with an apical ring, 40–60(–65) × 7–10(–11) µm (x = 47 ± 5.3 × 8.5 ± 1.1 µm, n = 10). **Ascospores** hyaline, fusoid to cylindrical, smooth, multiguttulate, often containing two guttules per cell, symmetrical to asymmetrical, straight to slightly curved, biseriate, with a median septum distinctly constricted, 12–14 × 3–4 µm (x = 13 ± 1 × 3.4 ± 0.3 µm, n = 30). **Conidiomata** pycnidial, hemispheric, somewhat erumpent, coated with white aerial mycelium, 1200–2500 µm, conidial masses extruding from the ostioles. **Conidiophores** hyaline, occasionally amber at the base, aseptate. **Conidiogenous cells** enteroblastic, polyphialidic, with 1–2 integrated loci, hyaline, verruculose, ampulliform to doliiform. **Conidia** hyaline, aseptate, ovoid to ellipsoid, apex obtuse, 3–4.5 × 2–2.5 µm (x = 3.6 ± 0.5 × 2.2 ± 0.2 µm, n = 30).

Fig. 3 Morphology of *Dendrostoma mali* from *Malus spectabilis*. a–c. Habit of pseudostromata on branches; d. transverse section of perithecia; e. longitudinal section through perithecia; f–j. ascii and ascospores; k. ascospores; l. m. conidiomata on PDA; n. conidiophores and conidiogenous cells; o. conidia. — Scale bars: a = 1 mm; b–e, l, m = 500 µm; f–k, n, o = 10 µm.
On PDA, cultures are white. Colonies are flat with regular edge; texture initially uniform, producing concentric circles after 3 wk with sparse conidiomata irregularly distributed on the agar surface.

Host & Distribution — On *Malus spectabilis* in China.

Material examined. **China**, Zhejiang Province, Hangzhou City, Linan, Tianmu Mountain, N30°19'02.62" E119°26'34.33", 320 m asl, on twigs and branches of *Malus spectabilis*, 21 Apr. 2017, Q. Yang & Z. Du (holotype CF 2017445; living ex-type culture CFCC 52102).

Notes — *Dendrostoma mali* is the type species of *Dendrostoma*, and presently is only known on *Malus spectabilis*. It can be distinguished from other known *Dendrostoma* spp. by the fusoid to cylindrical ascospores, and the ovoid to ellipsoid conidia with obtuse apices. *Dendrostoma mali* is assumed to be host specific which needs to be confirmed by additional studies.

*Dendrostoma quercinum* X.L. Fan & C.M. Tian, sp. nov. — MycoBank MB823989; Fig. 4

**Etymology.** Name derived from the host genus, *Quercus*.

*Pseudostromata* erumpent, consisting of an inconspicuous orange ectostromatic disc, semi-immersed to erumpent, causing a pustulate bark surface, 800–1500 µm diam. **Ectostromatic disc** flat or concave, orange, or brown to black, sometimes concealed by ostioles, surrounded by bark flaps, 500–1100 µm diam. **Central column** yellowish to brownish. **Stromatic zones** lacking. **Perithecia** conspicuous, umber to fuscous black, regularly scattered, surrounding the ectostromatic disc, 250–500 µm. **Ostioles** 3–8 per disc, flat in disc or sometimes slightly projecting, cylindrical, covered by an orange, umber to fuscous black crust, 100–150 µm diam. **Paraphyses** deliquescent. **Asci** fusoid, 8-spored, 2–3-seriate, with an apical ring, 53–70 × 9.5–10 µm (μ = 60.5 ± 4 × 9.8 ± 0.3 µm, n = 10). **Ascospores** hyaline, fusoid to cylindrical, smooth, multi-guttulate, often containing two guttules per cell, symmetrical to asymmetrical, straight to slightly curved, bicellular, with a median septum distinctly constricted, 16–22(–24) × 3–4 µm (μ = 20 ± 1.7 × 3.5 ± 0.4 µm, n = 30). **Conidiomata** pycnidial, hemispherical, somewhat erumpent, covered with cinnamon aerial mycelium, 900–2200 µm diam; conidial masses extruding from ostioles. **Conidiophores** hyaline, occasionally amber at the base, aseptate. **Conidigenous cells** enteroblastic, polyphialidic, with 1–2 integrated loci, hyaline, verruculose, ampulliform to doliform. **Conidia** hyaline, aseptate, fusoid, acute at each end, 10.5–14 × 2.5(–3) µm (μ = 12 ± 1 × 2.5 ± 0.2 µm, n = 30). On PDA, cultures are white, becoming hazel in the centre after 2 wk. The colonies are flat with regular edge; texture initially uniform, becoming dense in the centre after 2 wk, producing circular conidiomata at the margin of the compact centre.

Host & Distribution — On *Quercus acutissima* in China.

Materials examined (all on twigs and branches of *Quercus acutissima*).

**China**, Zhejiang Province, Hangzhou City, Hangzhou Botanical Garden, N30°15'13.25" E120°06'56.33", 49 m asl, 17 Apr. 2017, Q. Yang & Z. Du (holotype CF 2017461; living ex-type culture CFCC 52103); Zhejiang Province, Hangzhou City, Hangzhou Botanical Garden, N30°15'12.52", E120°06'57.02", 50 m asl, 17 Apr. 2017, Q. Yang & Z. Du (CF 2017462; living culture CFCC 52104); Hangzhou City, Hangzhou Botanical Garden, N30°15'13.77" E120°06'59.93", 46 m asl, 17 Apr. 2017, Q. Yang & Z. Du (CF 2017470; living culture CFCC 52105).

Notes — *Dendrostoma quercinum* can be distinguished from *D. mali* and *D. osmanthi* by its larger ascospores (16–24 × 3–4 µm), and DNA sequence data.

*Dendrostoma osmanthi* X.L. Fan & C.M. Tian, sp. nov. — MycoBank MB823990; Fig. 5

**Etymology.** Name derived from the host genus, *Osmanthus*.

*Pseudostromata* erumpent, consisting of an inconspicuous orange ectostromatic disc, semi-immersed to superficial, causing a pustulate bark surface, 1200–1400 µm diam. **Ectostromatic disc** flat or concave, orange, brown to black, sometimes concealed by ostioles, surrounded by bark flaps, 500–1100 µm diam. **Perithecia** conspicuous, umber to fuscous black, regularly scattered, surrounding the ectostromatic disc, 250–500 µm. **Ostioles** 3–8 per disc, flat in disc or sometimes slightly projecting, cylindrical, covered by an orange, umber to fuscous black crust, 100–150 µm diam. **Paraphyses** deliquescent. **Asci** fusoid, 8-spored, 2–3-seriate, with an apical ring, 53–70 × 9.5–10 µm (μ = 60.5 ± 4 × 9.8 ± 0.3 µm, n = 10). **Ascospores** hyaline, fusoid to cylindrical, smooth, multi-guttulate, often containing two guttules per cell, symmetrical to asymmetrical, straight to slightly curved, bicellular, with a median septum distinctly constricted, 16–22(–24) × 3–4 µm (μ = 20 ± 1.7 × 3.5 ± 0.4 µm, n = 30). **Conidiomata** pycnidial, hemispherical, somewhat erumpent, covered with cinnamon aerial mycelium, 900–2200 µm diam; conidial masses extruding from ostioles. **Conidiophores** hyaline, occasionally amber at the base, aseptate. **Conidigenous cells** enteroblastic, polyphialidic, with 1–2 integrated loci, hyaline, verruculose, ampulliform to doliform. **Conidia** hyaline, aseptate, fusoid, acute at each end, 10.5–14 × 2.5(–3) µm (μ = 12 ± 1 × 2.5 ± 0.2 µm, n = 30).
The bark surface of *Osmanthus fragrans* is pustulate, with the fungus appearing to be pathogenic to this host. *Dendrostoma osmanthi* is similar to *D. mali* but differs by having fusoid to cylindrical ascospores that are distinctly constricted at the median septum. The phylogenetic inferences indicated this species as an individual well-supported clade (MP/ML/BI = 100/99/1) in the genus *Dendrostoma*.

**Pseudomelanconidaceae** C.M. Tian & X.L. Fan, fam. nov. — MycoBank MB823991

Etymology. Name derived from the type genus, *Pseudomelanconis*.

Type genus. *Pseudomelanconis* C.M. Tian & X.L. Fan.

Asexual morph: melanconium-like. *Conidiomata* in bark, acer- vular, with an inconspicuous ectostromatic disc causing a more or less pustulate bark surface. *Central column* beneath the disc more or less conical and becoming pale brown or olive at maturity. The marginal part of ectostroma comprises conidio- phores and their basal cell layers. *Conidiophores* aseptate, unbranched, cylindrical, hyaline to pale brown, smooth-walled. *Conidiogenes* anellidic. *Conidia* ellipsoid to elongate pyriform, brown at maturity with hyaline sheath. Sexual morph: not observed.

Notes — The asexual morph of the new family *Pseudomelan- conidaceae* is similar to members of *Melanconiellaceae*, Melan-
conidaceae and Juglanconidaceae (Fan et al. 2016b, Voglmayr et al. 2017), but differs mainly by having conidiogenous cells with discreet annellations and an inconspicuous hyaline conidial sheath when mature. The phylogenetic inferences resolved this family as an individual group with well-supported value (MP/ML/BI = 100/100/1) from other families of Diaporthales.

**Pseudomelanconis** C.M. Tian & X.L. Fan, gen. nov. — Myco-Bank MB823992

**Etymology.** Name derived from pseudo- (false-, in Greek) and the genus name Melanconis.

**Type species.** *Pseudomelanconis caryae* C.M. Tian & X.L. Fan.

**Asexual morph.** melanconium-like. *Conidiomata* in bark, acer-vular, immersed in host bark to erumpent. *Ectostromatic disc* inconspicuous, causing a more or less putulose bark surface. *Central column* beneath the disc more or less conical. The marginal part of the central column comprises conidiophores and their basal cell layers. *Conidiophores* unbranched, aseptate, cylindrical, hyaline to pale brown, smooth-walled, sometimes reduced to conidiogenous cells. *Conidiogenous cells* annellic, sometimes with apical collarette. *Conidia* hyaline when immature, becoming brown at maturity, ellipsoid to oblong, multiguttulate, aseptate, (12.5–)13–15(–16) × 4–5 μm (T = 14 ± 1.1 × 4.5 ± 0.3 μm, n = 30), with distinct hyaline sheath, 0.5–1 μm diam, becoming inconspicuous when mature. *Conidial wall* smooth on the outer surface. **Sexual morph:** not observed.

**Pseudomelanconis caryae** C.M. Tian & X.L. Fan, sp. nov. — Myco-Bank MB823993; Fig. 6

**Etymology.** Named after the host genus from which it was isolated, Carya.

**Asexual morph.** melanconium-like. *Conidiomata* acervular, immersed in host bark to erumpent. *Ectostromatic disc* inconspicuous, causing a more or less putulose bark surface. *Central column* beneath the disc more or less conical. *Central column* beneath the disc more or less conical. *Conidiophores* unbranched, aseptate, cylindrical, hyaline to pale brown, smooth-walled, 14–30 μm. *Conidiogenous cells* annellidic, occasionally with distinct annellations and collarettes. *Conidia* hyaline when immature, becoming greyish sepia to olivaceous, ellipsoid to oblong, multiguttulate, aseptate, 5–7 μm (α = 10.5–14 × 5–7 μm) and beta conidia (2–2.5 × 0.8–1 μm; Wehmeyer 1941). *Sexual morph:** not observed.

**Materials examined** (all on twigs and branches of *Carya cathayensis*). **China.** Zhejiang Province, Hangzhou City, Linan, Tianmu Mountain, N30°18′49.14″ E119°26′30.44″, 285 m asl, 21 Apr. 2017, Q. Yang & Z. Du (holotype CF 2017466; living ex-type culture CFCC 52110); Zhejiang Province, Hangzhou City, Linan, Tianmu Mountain, N30°18′49.19″ E119°26′37.24″, 281 m asl, 21 Apr. 2017, Q. Yang & Z. Du (CF 2017467; living culture CFCC 52111); Hangzhou City, Linan, Tianmu Mountain, N30°18′48.77″ E119°26′36.56″, 287 m asl, 21 Apr. 2017, Q. Yang & Z. Du (CF 2017468; living culture CFCC 52112); Hangzhou City, Linan, Tianmu Mountain, N30°18′49.14″ E119°26′30.44″, 285 m asl, 21 Apr. 2017, Q. Yang & Z. Du (CF 2017469; living culture CFCC 52113).

Notes — *Pseudomelanconis caryae* is the type species of *Pseudomelanconis*, and only occurs on *Carya cathayensis* in China. Isolates were identified as *P. caryae* based on their characteristic morphology, host, and DNA phylogeny (MP/ML/BI = 100/100/1). *Juglanconis oblonga* is similar to *P. caryae*, but it can be distinguished by larger brown to blackish conidia (18–22.7 × 9.2–12), and distinctly integrated annellations, as well as DNA sequence data (Voglmayr et al. 2017). *Melanconis juglandis* var. *caryae* was recorded from *Carya cathayensis*, which was considered as a distinct species by Wehmeyer (1941). However, it differs from *P. caryae* primarily by hyaline alpha (10.5–14 × 5–7 μm) and beta conidia (2–2.5 × 0.8–1 μm; Wehmeyer 1941). Wehmeyer (1937) also transferred *Melanconia pallida* from *Carya* spp. to *Melanconis*, which differs from *P. caryae* in dark brown, subspherical to ovoid or oblong-cylindrical conidia (18–26.5 × 13.3–16.5 μm). Although *Pseudomelanconis* has acervular conidiomata covered by a pustulate conidial mass on the bark surface similar to *Melanconis* and *Juglanconis*, DNA sequence data confirmed them to represent a distinct phylogenetic lineage. Results of recent molecular phylogenetic investigations revealed a remarkably high diversity of corticolous melanconium-like fungi in *Diaporthales* (Fan et al. 2016b, Voglmayr et al. 2012, 2017).

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**Fig. 6** Morphology of *Pseudomelanconis caryae* from *Carya cathayensis*. a, b. Habit of conidiomata on branches; c. transverse section of conidioma; d. longitudinal section through conidioma; e–g. conidiophores and conidiogenous cells; h. conidia. — Scale bars: a = 1 mm; b–d = 500 μm; e–h = 10 μm.
**Synnemasporellaceae** X.L. Fan & J.D.P. Bezerra, fam. nov. — MycoBank MB823994

*Etymology.* Name derived from the type genus, *Synnemasporella.*

-Type genus.* Synnemasporella X.L. Fan & J.D.P. Bezerra.

*Pseudostromata* appearing upon the bark surface as pustules containing small groups of a few ostioles emergent through the adherent periderm, covered by a whitish pulverulence. *Stromatic zones* lacking, *Perithecia* spherical or flattened, with long necks, thickly clustered beneath the ecostromatic disks. *Asci* clavate. *Ascospores* biseriate, fusoid-ellipsoid, two-celled, hyaline, usually with a short, hyaline, bristle-like appendage at each end. *Conidiomata* synnematal or pycnidial. *Synnemata* determinate, parallel, consisting of slender, cylindrical black stalks and a spherical, capitulate, shiny black mass of conidia which was cut off from the ends of the numerous entwined hyphae of the stalk; conidiogenous cell zones concave. *Py-ncidia* with a central circular ostiole, hemispherical, immersed, somewhat erumpent. *Conidiophores* aggregated, straight to curved. *Conidiogenous cells* aggregated, hyaline, straight to curved, cylindrical. *Conidia* cylindrical to clavate, with a discrete hilum, smooth, pale brown.

-Notes—The new family **Synnemasporellaceae** is proposed to accommodate fungi without the typical characters of any of the two-celled, hyaline-spored, stromatic genera. Also, the synnematal and pycnidial conidiomata differ widely from melanconium-like fungi, having pale brown conidia with a distinct hilum (Wehmeyer 1933). The phylogenetic inferences resolved this family as a well-supported clade (MP/ML/BI = 98/98/1) between the families *Juglanconidiaceae* and *Apiosporisoridaceae.* Members of the new family differ from *Juglanconidiaceae* and *Apiosporisoridaceae* (Senanayake et al. 2017, Voglmayr et al. 2017) mainly in the type of the host plant association, disease symptoms, ascomatal and/or conidiomatal characters, shape of ascospores, conidiogenous cells and conidia, and distinct synnemata.

**Synnemasporella** X.L. Fan & J.D.P. Bezerra, *gen. nov.* — MycoBank MB823995

*Etymology.* Name derived from the synnematous conidiomata.

-Type species.* Synnemasporella toxicodendrai X.L. Fan & J.D.P. Bezerra.

**Sexual morph** (based on Wehmeyer 1933): *Pseudostromata* appearing upon the surface as pustules containing small groups of a few ostioles emergent through the adherent periderm, or as larger dense fascicles of elongate-cylindrical ostioles, erumpent through a whitish pulverulent disk, 0.3–1 mm diam, covered by a whitish pulverulence. *Stromatic zones* lacking, *Perithecia* spherical or flattened, with long slender necks, thickly clustered beneath the ecostromatic disks, 260–480 × 250–400 µm. *Asci* clavate, 47–65 × 5–8 µm. *Ascospores* biseriate, long fusoid-ellipsoid, two-celled, hyaline, constricted at the septum, 12–18 × 2.5–3 µm, and usually with a short, hyaline, bristle-like appendage at each end, 2–2.5 µm length. *Asexual morph:* *Conidiomata* synnematal or pycnidial. *Synnemata* long and determinate, growing from the host tissue, pale to brown, straight to curved, parallel, with convex and dark conidiogenous cells zone, and some host tissue at the base of synnema, 1100–1500 µm high, 200–400 µm diam. *Conidiophores* aggregated, aseptate, straight to curved, 20–30 µm. *Conidiogenous cells* aggregated, hyaline, straight to curved, cylindrical, arranged alongside one another at the end of the synnema, each producing one conidium. *Conidia* oblong-cylindrical, with a distinct hilum, smooth, multiguttulate, hyaline when young and becoming pale brown at maturity, 8–10(−11) × 3–3.5 µm (Σ = 9.3 ± 0.9 × 3.2 ± 0.3 µm, n = 30). *Pyecnidia* with a central circular ostiole, hemispherical, immersed, somewhat erumpent, containing an irregular one-chambered locule with black conidial mass, 700–1000 µm. *Conidiophores* aggregated, aseptate, straight to curved, 20–35 µm. *Conidiogenous cells* aggregated, hyaline, straight to curved, cylindrical, arranged alongside one another at the base of the pycnidia, each producing one conidium. *Conidia* ovoid to oblong-fusoid, one-celled, hyaline, multiguttulate, (6.5–)7–8.5(−9) × 2.5–3(−3.5) µm (Σ = 7.6 ± 0.6 × 3 ± 0.3 µm, n = 30).

On PDA, cultures are initially white, becoming straw on the margin after 3 wk. The colonies are fleshy with regular edge; texture initially uniform, producing concentric circle on the margin after 3 d; sterile.

-Host & Distribution — On *Rhus copallina, R. diversiloba, R. glabra, R. javanica, R. typhina* and *R. vernix* in Japan and USA (Wehmeyer 1933, Kobayashi 1970, Mejía et al. 2011), and on *R. chinensis* in China.

**Materials examined** (all on twigs and branches of *Rhus chinensis*). **CHINA,** Zhejiang Province, Hangzhou City, Linan, Xijing Mountain, N30°15’32.83” E119°43’30.73”, 47 m asl, 24 Apr. 2017, Q. Yang & Z. Du (CF 2017484; living culture CFCC 52094); Jiangxi Province, Dexing City, Phoenix Lake, N28°56’14.11” E117°35’32.84”, 41 m asl, 8 Apr. 2017, B. Cao (CF 2017401; living culture CFCC 52096); Jiangxi Province, Dexing City, Phoenix Lake, N28°56’15.20” E117°35’32.12”, 40 m asl, 8 Apr. 2017, B. Cao (CF 2017401; living culture CFCC 52096).

**Notes** — *Synnemasporella aculeans* is proposed as a new combination in the new genus *Synnemasporella* based on the description of *Cryptodiaporthe aculeans,* which was introduced producing perithecial ascomata, and an asexual morph producing sporodochial and/or pycnidial conidiomata (Wehmeyer 1933). Wehmeyer (1933) placed *C. aculeans* provisionally in *Cryptodiaporthe* and suggested this genus as a ‘heterogeneous group of species which will probably be segregated into several genera when the relationships of its species are better known’, highlighting that *C. aculeans* could be proposed as a new genus based on its atypical morphological features (see notes of *Synnemasporellaceae*). Sogonov et al. (2008) treated...
Recently, Senanayake et al. (2017) separated the genera *Plagiostoma* and *Cryptodiaporthe* in their phylogenetically inferences, and pointed out that *Gnomoniaceae* comprised 24 genera, including *Plagiostoma* and *Cryptodiaporthe*. In the current study, the phylogram indicated that our isolates clustered into the same clade (MP/ML/BI = 80/100/1) with the only available culture of *C. aculeans* (AR3878). Based on the phylogenetic inferences and morphological features, we transferred *C. aculeans* to the new genus *Synnemasporella*, as a new combination *S. aculeans*. *Synnemasporella aculeans* is similar to *S. toxicodendri*, but differs from it in having shorter synnemata (1100–1500 µm vs 1200–1800 µm), a convex conidiogenous cells zone on top of synnema, and larger, oblong-cylindrical conidia (8–11 × 3–3.5 µm).

This species also represents two types of conidiomata in *Diaporthales*, namely pycnidia and synnemata. This uncommon phenomenon was also recorded by Wehmeyer (1933), who observed the production of sporodochia on twigs of *Rhus*. Wehmeyer (1933) also reported the production of conidiomata when cultures were grown on agar, ‘showing all intergradations between true pycnidia and true sporodochia’.

**Synnemasporella toxicodendri** X.L. Fan & J.D.P. Bezerra, sp. nov. — MycoBank MB823997; Fig. 8

**Etymology.** Name derived from the host genus, *Toxicodendron*.

**Conidiomata** synnematal. *Synnemasporella* long and determinate, growing from host tissue, pale to brown, straight to curved, parallel, with flat to slightly concave and dark conidiogenous cells zone and some host tissue at the base of synnema, 1200–1800 µm high, 150–300 µm diam. **Conidiophores** aggregated, aseptate, straight to curved, reduced to conidiogenous cells, 20–30 µm. **Conidiogenous cells** aggregated, hyaline, straight to curved, cylindrical, arranged adjacent to one another at the end of the synnema, producing one conidium each. **Conidia** cylindrical to oblong-cylindrical, with a discrete hilum, smooth, multiguttulate, pale brown, 6–8 × 2.5–4 µm (X = 7.3 ± 0.6 x 3.1 ± 0.3 µm, n = 30). **Sexual morph:** not observed.

On PDA, cultures are initially white, becoming sepia on the bottom after 3 d. The colonies are felty with an irregular edge; texture initially uniform, producing concentric circles after 3 wk; sterile.

**Host & Distribution.** From *Toxicodendron sylvestre* in China.

**Materials examined** (all on twigs and branches of *Toxicodendron sylvestre*). 中国, Zhejiang Province, Hangzhou City, Linan, Xijing Mountain, N30°15'32.84" E119°43'31.21", 54 m asl, 22 Apr. 2017, Q. Yang & Z. Du (holotype CF 2017481; living ex-type culture CFCC 52097); Zhejiang Province, Hangzhou City, Linan, Xijing Mountain, N30°15'32.21" E119°43'31.55", 51 m asl, 22 Apr. 2017, Q. Yang & Z. Du (CF 2017483; living culture CFCC 52099);
Notes — Structures of *S. toxicodendri* were observed growing on diseased wood of *Toxicodendron sylvestre* in China, and so far, only occurs on *T. sylvestre*. Morphologically, it can be distinguished from *S. aculeans* and other genera in *Diaporthales* because it is characterised by the higher synnemata (1200–1800 μm) growing from the host tissue, with flat to slightly concave and dark conidiogenous cells zone on the top. The sexual morph of this species is not known and further collections are required to resolve its life cycle.

DISCUSSION

In this study we propose three new families, namely *Diaporthostomataceae*, *Pseudomelanconidaceae*, *Synnemasporellaceae*, and a new genus, *Dendrostoma* (*Erythrogloeaceae*), including three new species. The new materials studied here were collected in Zhejiang Province, China. This province was chiefly selected due to Tianmu Mountain, which is considered as a biodiversity hotspot with a high diversity for forest species (Zhou 1995). In the current study, all specimens were collected from symptomatic branches and twigs associated with canker or dieback disease of *Anacardiacaeae* (*Rhus chinensis*, *Toxicodendron sylvestre*), *Fagaceae* (*Quercus acutissima*), *Juglandaceae* (*Carya cathayensis*), *Laureaceae* (*Machilus leptophylla*), *Oleaceae* (*Osmanthus fragrans*) and *Rosaceae* (*Malus spectabilis*), suggesting that many additional undiscovered species of diaporthalean fungi exist in China.

The classification of *Diaporthales* presented here integrates results from prior analyses (Castlebury et al. 2002, Rossman et al. 2007) and discoveries of new taxa from many other research groups (Cheewangkoon et al. 2010, Crous et al. 2012, 2015, Su et al. 2015, Norphanchon et al. 2016, Senanayake et al. 2017, Voglmayr et al. 2017). *Diaporthales* are mostly characterised by diaporthalean perithecia with an elongate beak and unitunicate asci with a characteristic refractive apical annulus when mature (Rossman et al. 2007). However, these characters are difficult to fully study in vivo due to the absence of various morphological morphs. As a result, the family concepts have thus been unstable, with many species being transferred from one genus or family to another (Rossman et al. 2007, Hongsanan et al. 2017, Senanayake et al. 2017). Based on newly generated molecular data, the current systematic framework provides good support for this order and related families (Castlebury et al. 2002, Rossman et al. 2007, Crous et al. 2015, Senanayake et al. 2017, Voglmayr et al. 2017). The current study revised the Diaporthales and accepted 25 families in the order (Table 2). However, some nodes remain weakly supported, and genera such as *Diaporthella* and *Phaeoappendispora* require further collection and study (Senanayake et al. 2017).

As the morphological features in *Diaporthales* are highly diverse, phylogenetic studies have been useful to elucidate the diversity in this group, and the inclusion or exclusion of taxa in this order. In this study we proposed a new genus in *Erythrogloeaceae*, namely *Dendrostoma*, which is characterised by typical diaporthalean perithecia with clavate asci, and fusoid to cylindrical, bicalcareous ascospores. The family *Erythrogloeaceae* was recently proposed by Senanayake et al. (2017) to accommodate *Chrysocrypta*, *Disculoides* and *Erythroglueum* based on morphological features and phylogenetic analyses. Members of this family are mainly characterised by acervular idiomata, hyaline to olivaceous conidia, and the presence of macro- and microconidia. Although Senanayake et al. (2017) did not observe any sexual morph in this family, we did collect a sexual morph in the present study, and thus emended the family description accordingly.

The new family *Diaporthostomataceae* is introduced here based on phylogenetic inferences and morphology of its members, which are mainly characterised by conical and discrete perithecia, and bicalcarate, fusoid ascospores. The new family is morphologically distinct from its sister family *Diaporthosporellaceae*, which is also distinguished from other diaporthalean families by irregularly uniseriate, allantoid or subreniform ascospores, phialidic conidiophores, and cylindrical to ellipsoidal, aseptate conidia (Yang et al. 2018). Members of *Diaporthosporellaceae* are known from twigs and branches of *Cercis chinensis* (Yang et al. 2018), and representatives of *Diaporthostomataceae* occur on *Machilus leptophylla*, both occurring in China.

*Pseudomelanconidaceae* is described here (on *Carya cathayensis* in China) based on phylogenetic inferences, and on few morphological features which distinguish it from members of *Melanconium*-related *Melanconidiaceae*, *Melanconidaceae* and *Juglanconidaceae*. Senanayake et al. (2017) introduced...
Synnemasporella with long necks, based on the original description of Wehmeyer (1933), and included a single genus, Melanconis, which is characterised by perithecial ascomata having 8-spored asci, hyaline, ellipsoid, 1-septate ascospores, and acervular conidiomata with hyaline to brown, ellipsoid or subglobose conidia. Members of this family include saprobic and plant pathogenic species in North America and Europe (Senanayake et al. 2017). Juglanconidaceae was recently revised by Senanayake et al. (2017), and included a single genus, Melanconis, which is closely related to perithecial ascomata occurring on Eucalyptus leaves. Fungal Diversity 44: 89–105.

The molecular phylogenetic analyses also revealed another well-supported family, Synnemasporellaceae, which is closely related to Juglanconidaceae (Fig. 1). We identified one species of this clade as Cryptodiaporthe aculeans based on the ambiguous asexual features of this species, which can produce synnemata and pycnidia. Interestingly, C. aculeans was 'arbitrarily placed in the genus Cryptodiaporthe' by Wehmeyer (1933), who also suggested that this species could be described as a new genus 'since it does not show the typical characters of any of the two-celled, hyaline-spored, stromata genera'. Recent studies, however, did not include Cryptodiaporthe aculeans in their phylogenetic analyses (Sogonov et al. 2008, Mejía et al. 2011, Senanayake et al. 2017). In the present study, this fungus was transferred to Synnemasporella as a new combination, based on the original description of Wehmeyer (1933), and the only sequenced culture (AR3878) available in GenBank. Synnemasporellaceae comprises fungi distinguished by the presence of spherical or flattened perithecia with long necks, clavate ascii, fusoid-ellipsoid, two-celled, hyaline ascospores, usually with a short, hyaline, bristle-like appendage at each end, and synnematal and/or pycnidial conidiomata producing cylindrical to clavate, smooth, pale brown conidia.

As shown in this study, future studies addressing the family-level organization of the Diaporthales should routinely include data for protein-coding genes, especially rpβ2 and tef1-α. It is hoped that the classification proposed here will also provide an updated phylogenetic framework that will facilitate further revision of the Diaporthales.

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