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Families of *Diaporthales* based on morphological and phylogenetic evidence

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Abstract: *Diaporthales* is an important ascomycetous order comprising phytopathogenic, saprobic, and endophytic fungi, but interfamilial taxonomic relationships are still ambiguous. Despite its cosmopolitan distribution and high diversity with distinctive morphologies, this order has received relativelyiaceae, *Macrohilaceae*, *Melanconidaceae*, *Pseudoplagiostomaceae*, *Schizoparmaceae*, *Stilbosporaceae* and *Sydowiellaceae*. Taxonomic uncertainties among genera are also clarified and recurrent discrepancies in the taxonomic position of families within the *Diaporthales* are discussed. An updated outline and key to families and genera of the order is presented.

Key words: Multi-gene DNA phylogeny, New taxonomic arrangement, Phytopathogenic fungi, Sordariomycetes, Systematics.

Taxonomic novelties: New families: *Apiosporopsidaceae* Senan. Maharachch. & K.D. Hyde, *Apoharknessiaceae* Senan. Maharachch. & K.D. Hyde, *Asterosporiaceae* Senan. Maharachch. & K.D. Hyde, *Auratiopycnidiellaceae* Senan. Maharachch. & K.D. Hyde, *Erythrogloeaceae* Senan. Maharachch. & K.D. Hyde, *Melanconiellaceae* Senan. Maharachch. & K.D. Hyde, *Prosopidicolaceae* Senan. & K.D. Hyde; **New genera:** *Marsupiomyces* Senan. & K.D. Hyde, *Microascospora* Senan., Camporesi & K.D. Hyde, *Phaeoappendicospora* Senan., Q.R. Li & K.D. Hyde, *Paradiaporthe* Senan., Camporesi, & K.D. Hyde, *Hyaliappendispora* Senan., Camporesi & K.D. Hyde, *Chiangraiomyces* Senan. & K.D. Hyde; **New species:** *Chiangraiomyces bauhiniae* Senan. & K.D. Hyde, *Coniella pseudokoreana* Senan., Tangthir. & K.D. Hyde, *Cytospora centrivillosa* Senan., Camporesi & K.D. Hyde, *Cytospora junipericola* Senan., Camporesi & K.D. Hyde, *Cytospora quercicola* Senan., Camporesi & K.D. Hyde, *Cytospora rosae* Senan., Camporesi & K.D. Hyde, *Cytospora fraxinigena* Senan., Camporesi & K.D. Hyde, *Diaporthe litoricola* Senan., E.B.G. Jones & K.D. Hyde, *Ditopella biseptata* R.H. Perera, Senan., Camporesi & K.D. Hyde, *Gnomoniopsis agrimoniae* Senan., Camporesi & K.D. Hyde, *Hyaliappendispora galii* Senan., Camporesi & K.D. Hyde, *Marsupiomyces epidermoidea* R.H. Perera, Senan., Bulgakov & K.D. Hyde, *Marsupiomyces quercina* Senan., Camporesi & K.D. Hyde, *Melanconis italica* Senan., Camporesi & K.D. Hyde, *Microascospora rubi* Senan., Camporesi & K.D. Hyde, *Paradiaporthe artemisiae* Senan., Camporesi & K.D. Hyde, *Phaeoappendicospora thailandensis* Senan., Q.R. Li & K.D. Hyde, *Plagiostoma jonesii* Senan., & K.D. Hyde, *Plagiostoma salicicola* Senan., Camporesi & K.D. Hyde, *Sydowiella urticicola* Senan., Camporesi & K.D. Hyde, *Tubakia thailandensis* Senan., Tangthir., K.D. Hyde; **New combinations:** *Coryneum arausiaca* (Fabre) Senan., Maharachch. & K.D. Hyde, *Microascospora fragariae* (F. Stevens & Peterson) Senan., Maharachch. & K.D. Hyde.

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INTRODUCTION

The *Diaporthales* is a distinct order in the subclass *Diaporthomycetidae* (*Sordariomycetes*) and it includes pathogens, saprobes and endophytes, with no known coprophilous, hypersaprobes or mycophylic species (Barr 1978, Rossman *et al.* 2007, Vasilyeva *et al.* 2007, Maharachchikumbura *et al.* 2015, 2016). Taxa of this order inhabit a wide diversity of hosts and substrates, including most economically and ecologically important trees and crops, soil and living animal and human tissues (Barr 1978, Gryzenhout *et al.* 2006c). Species in *Diaporthales* form solitary or aggregated, immersed to erumpent, rarely superficial, orange, brown to black perithecial ascomata, with short or long necks, that are located in stromatic tissues or substrates, with a centrum (or hamathecium) lacking or with few paraphyses (Alexopoulos & Mims 1978, Barr 1978, Castlebury *et al.* 2002). Asci are unitunicate with a conspicuous refractive ring (Hawksworth *et al.* 1995, Rossman *et al.* 2007). Ascospore morphology is diverse, ranging from short to elongate and aseptate or septate with hyaline or pigmented walls. The asexual morphs of *Diaporthales* are generally coelomycetous (Rossman *et al.* 2007), producing acervuli or pycnidial conidiomata, with or without a well-developed stroma. Conidiogenesis is phialidic or rarely annellidic and conidia are usually unicellular or 1-septate (Rossman *et al.* 2007).

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Studies in Mycology

Fungal taxa placed in "*Diaporthaceae sensu lato*" were divided into two groups (von Höhnel 1917) as "Eu-Diaportheen", to accommodate genera without allantoid ascospores and "Valseen" to accommodate genera with allantoid ascospores. Nannfeldt (1932) introduced the order *Diaporthales* to accommodate von Höhnel's Eu-Diaportheen group. Luttrell (1951) described *Diaporthales* as an order comprising species that have a "*Diaporthe*-type centrum" and "*Endothia*-type ascus". Chadefaud (1960) analysed characters of stromatic tissues in diaporthoid taxa and recognised families as *Diaporthaceae* or *Cytosporaceae* (= *Valsaceae*), *Melanconidaceae* and *Gnomoniaceae*. Wehmeyer (1975) classified the *Diaporthales* to include three families: *Diaporthaceae*, *Gnomoniaceae* and *Cytosporaceae*. Barr (1978) revised the order *Diaporthales* accepting *Gnomoniaceae* and *Cytosporaceae* in the suborder *Gnomoniineae*. *Melanconidaceae* and *Pseudovalsaceae* were accommodated in the suborder *Melanconidineae*. To differentiate genera, Barr (1978) used characters such as presence or absence of stromata, stromatic development and tissue types, the position of the perithecia and perithecial necks relative to the substrate, as well as ascospore shape; and Monod (1983) distinguished genera within *Gnomoniaceae* based on characters of the stromatic tissues, asexual morphs and ascospores. Three families were recognised in *Diaporthales* by Eriksson (2001), including *Cytosporaceae*, *Melanconidaceae* and *Vialaeaceae*. Based on analysis of LSU nrDNA sequence data, Castlebury *et al.* (2002) accepted *Diaporthaceae*, *Gnomoniaceae*, *Melanconidaceae* and *Cytosporaceae* in *Diaporthales*. *Gnomoniaceae* was revised by several recent studies and new taxa were introduced (Sogonov *et al.* 2008, Walker *et al.* 2010, 2012, Mejía1 *et al.* 2011). Castlebury *et al.* (2002) did not confirm *Vialaeaceae* as a family in *Diaporthales* and therefore excluded it from *Diaporthales*. Réblová *et al.* (2004) introduced *Togniniaceae* to this order based on small subunit (SSU) nrDNA; however, Mostert *et al.* (2006) concluded that its placement was ambiguous based on large subunit (LSU) nrDNA. Maharachchikumbura *et al.* (2015) excluded *Togniniaceae* from *Diaporthales* and accommodated it in *Togniniales*. Gryzenhout *et al.* (2006c) introduced the *Cryphonectria–Endothia* complex as the family *Cryphonectriaceae*. *Sydowiellaceae* and the *Schizoparme–Pilidiella* complex with the genus *Coniella* were introduced as *Schizoparmaceae* in *Diaporthales* (Rossman *et al.* 2007, Alvarez *et al.* 2016). *Harknessiaceae* was introduced into *Diaporthales* accommodating *Harknessia* with wuestneia-like sexual morphs (Crous *et al.* 2012b). *Pseudoplagiostomaceae* was introduced by Cheewangkoon *et al.* (2010) to accommodate *Pseudoplagiostoma*. Voglmayr & Jaklitsch (2014) resurrected *Stilbosporaceae* in *Diaporthales* based on phylogenetic analysis of LSU nrDNA sequence data and transferred the genera *Stegonsporium* and *Stilbospora* to this family. *Macrohilaceae* was introduced by Crous *et al.* (2015), based on an analysis of LSU nrDNA to accommodate *Macrohilum*. Suetrong *et al.* (2015) introduced *Tirisporellaceae* into *Diaporthales*; however, Jones *et al.* (2015) excluded this family from *Diaporthales*. Norphanphoun *et al.* (2016) introduced *Lamproconiaceae* to accommodate *Lamproconium* and *Hercospora*. *Juglanconidaceae* was introduced in the *Diaporthales* by Voglmayr *et al.* (2017). However, molecular data suggest that additional families still remain to be elucidated (Gryzenhout *et al.* 2006c, Crous *et al.* 2012a, 2015, Voglmayr *et al.* 2017). Currently there are 14 families accepted in the *Diaporthales*.

Given the taxonomic discrepancies within *Diaporthales*, the present study uses a combined taxonomic approach based on morphology and DNA sequence analyses of the partial 28S nrDNA (LSU), the internal transcribed spacer regions and intervening 5.8S nrDNA (ITS), DNA-directed RNA polymerase II second largest subunit (*rpb2*), and translation elongation factor 1-alpha (*tef1*) gene regions to investigate phylogenetic relationships of all genera in *Diaporthales* to update their classification. All taxonomic novelties and present taxonomic families are redescribed and illustrated where necessary. We also present new data on each family to provide a better taxonomic understanding.

MATERIALS AND METHODS

Isolates and specimens

Specimens were collected from Germany, Italy, Russia, Thailand and the UK. They were placed in paper bags and collection details noted. Specimens were brought to the laboratory in Ziplock plastic bags and examined with a Motic SMZ 168 stereomicroscope. Rehydrated fruiting bodies were used to observe morphological characteristics of ascomata, asci, ascospores and other tissues and characters were photographed with a Canon 550D digital camera fitted to the Nikon ECLIPSE 80i compound microscope. Photomicrographs were arranged with Adobe Photoshop v. CS6 and all measurements were made with Tarosoft v. 0.9.0.7. Specimens were preserved and are deposited at the BBH and MFLU fungaria. Taxonomic novelties and descriptions were deposited in MycoBank (Crous *et al.* 2004), and new species were established using modern criteria and standards (Taylor *et al.* 2000, Seifert & Rossman 2010, Jeewon & Hyde 2016).

Sporocarps were removed from the substrate using a sterilised needle and placed in a few drops of sterilised distilled water on a sterilised cavity slide and a spore suspension was prepared as described in Chomnunti *et al.* (2014). Germinating ascospores were aseptically transferred to Petri dishes containing Potato Dextrose Agar (PDA) or Malt Extract Agar (MEA) (Crous *et al.* 2009). Colonies were photographed and characters were noted. Colony colour on PDA and MEA was determined with the colour charts of Rayner (1970). Living cultures are deposited at Mae Fah Luang University (MFLU) and the Westerdijk Fungal Biodiversity Institute (CBS) culture collections. Autoclaved pine needles were placed on water agar (WA) to observe conidiomatal development and sporulating (Crous *et al.* 2009).

Types and other relevant authentic specimens were loaned from accessible fungaria [New York State Museum (NY), Naturhistorisches Museum Wien (W), Swedish Museum of Natural History (S), Royal Botanic Gardens, Kew (K), Universität Wien (WU)]. A small part of the fungarium specimen was cut and rehydrated in water or 5 % KOH. Micro-morphological characters were observed from rehydrated ascomata and photography was done as previously described.