Coelomycetous *Dothideomycetes* with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*

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Abstract: The taxonomy of the coelomycetes has undergone dramatic changes in recent years, but remains controversial due to the high number of taxa involved, their poor morphological differentiation, the rare occurrence of the sexual morphs, and rapid loss of fertility *in vitro*. In the present study, we revisited the families *Cucurbitariaceae* and *Didymellaceae* (*Pleosporales, Dothideomycetes*), which include numerous plant pathogens, endophytic species associated with a wide host range, and saprobes. The taxonomy of two of the most relevant genera, i.e. *Phoma* and *Pyrenochaeta*, remains ambiguous after several phylogenetic studies, and needs further revision. We have studied a total of 143 strains of coelomycetes from clinical or environmental origin, by combining the LSU, ITS, *tub*2 and *rpb*2 sequences for a multilocus analysis and a detailed morphological comparison. The resulting phylogenetic tree revealed that some fungi previously considered as members of *Cucurbitariaceae* represented five different families, and four of them, *Neopyrenochaetaceae*, *Parapyrenochaetaceae*, *Pseudopyrenochaetaceae* and *Pyrenochaetopsidaceae*, are proposed here as new. Furthermore, 13 new genera, 28 new species, and 20 new combinations are proposed within the *Pleosporineae*. Moreover, four new typifications are introduced to stabilise the taxonomy of these fungi.

Key words: *Cucurbitariaceae*, *Didymellaceae*, Multigene phylogeny, New taxa, *Phoma*, *Pleosporineae*, *Pleosporales*, *Pyrenochaeta*, *Pyrenochaetopsis*, Taxonomy.
Taxonomic novelties: New families: *Neopyrenochaetaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Parapyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaetaceae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pyrenochaetopsidaceae* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel; **New genera:** *Allocucurbitaria* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Cumuliphoma* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ectophoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Juxtiphoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopyrenochaetopsis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Paracucurbitaria* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Parapyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Remotididymella* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Similiphoma* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Vacuiphoma* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Xenopyrenochaetopsis* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano; **New species:** *Allocucurbitaria botulispora* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Allophoma cylindrispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Cumuliphoma indica* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Cu. pneumoniae* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Didymella brunneospora* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *D. keratinophila* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Epicoccum catenisporum* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ep. keratinophilum* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Ep. ovisporum* Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ep. pneumoniae* Valenzuela-Lopez, Stchigel, Guarro & Cano, *Neoascochyta cylindrispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Neoa. tardicrescens* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neocucurbitaria aquatica* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu. irregularis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Neopyrenochaeta fragariae* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopyrenochaetopsis hominis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Nothophoma variabilis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Paracucurbitaria italica* Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta terrestris* Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Pyrenochaetopsis americana* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. botulispora* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. confluens* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. globosa* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. paucisetosa* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Py. setosissima* Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Py. uberiformis* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Remotididymella anthropophila* Valenzuela-Lopez, Cano, Guarro & Stchigel, *Vacuiphoma oculihominis* Valenzuela-Lopez, Stchigel, Guarro & Cano; **New combinations:** *Cumuliphoma omnivirens* (Aveskamp *et al.*) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Ectophoma multirostrata* (P.N. Mathur *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Ec. pomi* (Horne) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Epicoccum proteae* (Crous) Valenzuela-Lopez, Stchigel, Crous, Guarro & Cano, *Juxtiphoma eupyrena* (Sacc.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Neocucurbitaria cava* (Schulzer) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu. hakeae* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neocu. keratinophila* (Verkley *et al.*) Valenzuela-Lopez, Stchigel, Guarro & Cano, *Neopyrenochaeta acicola* (Moug. & Lév.) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopy. inflorescentiae* (Crous *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Neopy. telephoni* (Rohit Sharma *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Paracucurbitaria corni* (Bat. & A.F. Vital) Valenzuela-Lopez, Stchigel, Guarro & Cano, *Parapyrenochaeta acaciae* (Crous *et al.*) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Parapy. protearum* (Crous) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Pseudopyrenochaeta lycopersici* (R.W. Schneid. & Gerlach) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano, *Remotididymella destructiva* (Plowr.) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Similiphoma crystallifera* (Gruyter *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Vacuiphoma bulgarica* (Aveskamp *et al.*) Valenzuela-Lopez, Cano, Crous, Guarro & Stchigel, *Xenodidymella saxea* (Aveskamp *et al.*) Valenzuela-Lopez, Crous, Cano, Guarro & Stchigel, *Xenopyrenochaetopsis pratorum* (P.R. Johnst. & Boerema) Valenzuela-Lopez, Crous, Stchigel, Guarro & Cano.

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INTRODUCTION

The *Pleosporales* is the largest order of the class *Dothideomycetes* (phylum *Ascomycota*), encompassing more than 4 700 species distributed over 332 genera, and 53 families (Kirk *et al.* 2008, Zhang *et al.* 2009, 2012, Ariyawansa *et al.* 2013, Hyde *et al.* 2013, Amaradasa *et al.* 2014, Trakunyingcharoen *et al.* 2014, Wijayawardene *et al.* 2014, Crous *et al.* 2015a, Sharma *et al.* 2015, Tanaka *et al.* 2015, Jaklitsch *et al.* 2016, Jaklitsch & Voglmayr 2016, Wanasinghe *et al.* 2016, Crous & Groenewald 2017, Hashimoto *et al.* 2017, Hernández-Restrepo *et al.* 2017). These fungi are characterised by the production of pseudothecial ascomata (mostly globose and usually papillate) consisting of a peridial wall composed by several layers of cells, within which the fissitunicate (bitunicate) asci are produced amidst a persistent hamathecium (the vegetative structures inside an ascoma) (Jaklitsch & Voglmayr 2016, Jaklitsch *et al.* 2017, Zhang *et al.* 2009, 2012) and ascospores, which are mostly septate but variable in shape and pigmentation. The asexual morphs of the *Pleosporales* are characterised by conidia produced within discrete sporocarps (conidiomata), and sometimes conidia are generated on conidiophores produced on mycelium. *Phoma* and its relatives are the most common pleosporalean asexual morphs and are characterised by the presence of pycnidia (globose to pyriform conidiomata from which the conidia arise throughout an apical opening) (de Gruyter *et al.* 2009, 2010, Aveskamp *et al.* 2010, Chen *et al.* 2015). *Pleosporales* are mainly saprobic on plant debris, epiphytic, endophytic or parasitic of living plants, fungi and insects, or mycobionts in lichens (Kruys *et al.* 2006, Aveskamp *et al.* 2008, 2010, de Gruyter *et al.* 2009, Zhang *et al.* 2009, 2012, Lawrey *et al.* 2012, Kocakaya *et al.* 2015). These fungi can also infect humans (Punithalingam 1979, Ahmed *et al.* 2014, 2015, 2017, Borman *et al.* 2016, Valenzuela-Lopez *et al.* 2016).

Modern phylogenetic studies support the division of the *Pleosporales* into the suborders *Pleosporineae* and *Massarineae* (Zhang *et al.* 2009, 2012, Hyde *et al.* 2013, Tanaka *et al.* 2015). The former includes nine families, i.e. *Coniothyriaceae*, *Cucurbitariaceae*, *Didymellaceae*, *Dothidotthiaceae*, *Halojulellaceae*, *Leptosphaeriaceae*, *Neophaeosphaeriaceae*, *Phaeosphaeriaceae*, *Pleosporaceae* and *Shiraiaceae* (Zhang *et al.* 2012, de Gruyter *et al.* 2013, Ariyawansa *et al.* 2013, 2015b, Liu *et al.* 2013), which encompass plant pathogens of economic importance including the well-known genera such as *Alternaria*, *Ascochyta*, *Bipolaris*, *Didymella* and *Leptosphaeria* (Zhang *et al.* 2012, Ariyawansa *et al.* 2013, de Gruyter *et al.* 2013, Liu *et al.* 2013, Woudenberg *et al.* 2013). Recently, Tanaka *et al.* (2015) revised the suborder *Massarineae* and accepted 12 families; however, more studies are needed for a better understanding of their phylogenetic relationships. Numerous species of *Pleosporales* are relatively common in clinical samples, most of which belong to the families *Cucurbitariaceae* and *Didymellaceae* (Valenzuela-Lopez *et al.* 2016). *Cucurbitariaceae* is still a poorly known family, which was erected by Winter (1885) with *Cucurbitaria* as the type genus, and characterised by ostiolate ascomata aggregated on a basal pseudostromatic structure, hamathecium composed of wide persistent filaments, fissitunicate, cylindrical to cylindrical-clavate asci and dark, phragmosporous or muriform ascospores. In the last revision of *Cucurbitariaceae*, four sexual genera (*Cucurbitaria*, *Curreya*, *Rhytidiella* and *Syncarpella*) and two asexual genera (*Pyrenochaeta* and *Pyrenochaetopsis*) were accepted (Doilom *et al.* 2013). The latter two genera are characterised by phoma-like, setose pycnidia, and hyaline, aseptate conidia (de Gruyter *et al.* 2010, 2013). Recently, Jaklitsch & Voglmayr (2017) demonstrated that some species of *Cucurbitaria*, such as *C. obducens*, *C. piceae* (both producing muriform ascospores) and *C. rhododendri* (with phragmospores), belong to three different genera of *Melanommataceae*. Wanasinghe *et al.* (2017b) proposed *Neocucurbitaria*, characterised by solitary ascomata, the presence of periphyses and muriform ascospores, as a new genus of *Cucurbitariaceae*. However, the current members of this family need to be re-evaluated, including their asexual morphs.

The family *Didymellaceae* also includes economically important plant pathogens, such as the causal agents of blackleg and ascochyta blight (Rouxel & Balesdent 2005, McDonald & Peck 2009, Salam *et al.* 2011, de Gruyter *et al.* 2013), but also diverse endophytic, fungicolous and lichenicolous taxa belong to this fungal group (Aveskamp *et al.* 2010), whereas a few members are known as pathogens of humans (de Hoog *et al.* 2011). This family was established by de Gruyter *et al.* (2009) and embraces the species traditionally classified in the genera *Ascochyta*, *Didymella* and *Phoma*. However, *Phoma* is one of the largest and most polyphyletic fungal genera (with more than 3 000 names recorded) with species occurring in more than 25 families (http://www.indexfungorum.org).

Zhang *et al.* (2009), included *Didymellaceae* in their study and accepted the sexual genera *Didymella*, *Leptosphaerulina*, *Macroventuria*, *Monascostroma* and *Platychora*. In general, these genera are characterised by dark pseudothecial ascomata, filamentous pseudoparaphyses, 8-spored, fissitunicate, clavate to saccate asci, and hyaline, 1-septate, fusiform to biconical ascospores; with the only exception being *Leptosphaerulina*, which has hyaline to brown, ellipsoid, cylindrical or oblong, phragmosporous or muriformly septate ascospores, which also lack pseudoparaphyses. Several studies have tried to resolve the taxonomy of the asexual morphs of the *Didymellaceae*, especially *Phoma* and its relatives, with more or less success. Subsequently, de Gruyter *et al.* (2010) transferred several species of *Phoma* to *Pyrenochaetopsis* (*Cucurbitariaceae*), *Neosetophoma* and *Setophoma* (*Phaeosphaeriaceae*), and resurrected the genus *Paraphoma* (*Phaeosphaeriaceae*). The study by Aveskamp *et al.* (2010), based on the sequences of four loci, revealed that the subdivision of *Phoma* in sections (Boerema *et al.* 2004) was phylogenetically inconsistent, and they thus proposed *Boeremia* to accommodate species morphologically close to *Phoma exigua*, while species of *Phoma* section *Sclerophomella* were transferred to *Epicoccum* and *Peyronellaea*. Furthermore, de Gruyter *et al.* (2013) transferred some species of *Phoma* sections *Plenodomus* and *Heterospora* to the *Leptosphaeriaceae* and some from *Phoma* section *Pilosa* and *Ascochyta* to *Pleosporaceae*. Recently, Chen *et al.* (2015) proposed nine genera (*Allophoma*, *Calophoma*, *Heterophoma*, *Neoascochyta*, *Neodidymelliopsis*, *Nothophoma*, *Paraboeremia*, *Phomatodes* and *Xenodidymella*) in *Didymellaceae*, transferred *Microsphaeropsis* (*Didymellaceae*) to the family *Microsphaeropsidaceae*, and restricted *Phoma* to *Phoma herbarum* (Chen *et al.* 2017). Other authors have added the genera *Briansuttonomyces*, *Didymellocamarosporium*, *Heracleicola*, *Neodidymella*, *Neomicrosphaeropsis* and *Pseudoascochyta* to *Didymellaceae* (Ariyawansa *et al.* 2015a, Crous & Groenewald 2016, Crous *et al.* 2016a, Thambugala *et al.* 2016, Wijayawardene *et al.* 2016). However, the genera *Didymellocamarosporium*, *Heracleicola* and *Neodidymella* were studied by