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journal homepage: www.elsevier.com/locate/funbio

They seldom occur alone

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ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form
16 May 2016
Accepted 19 May 2016
Available online 30 May 2016
Corresponding Editor:
Ana Crespo

Keywords:
Dothideales
Helotiales
Multigene phylogeny
Systematics

ABSTRACT

Species of *Coleophoma* have been reported as plant pathogenic, saprobic or endophytic on a wide host range. The genus is characterised by having pycnidial conidiomata, phialidic conidiogenous cells intermingled among paraphyses, and cylindrical conidia. *Coleophoma* has had a confusing taxonomic history with numerous synonyms, and its phylogeny has remained unresolved. The aim of the present study was to use a polyphasic approach incorporating morphology, ecology, and molecular data of the partial large subunit of nrDNA (LSU), the internal transcribed spacer region with intervening 5.8S nrDNA (ITS), partial β-tubulin (*tub2*), and translation elongation factor 1-alpha (*tef1*) gene sequences to resolve its taxonomy and phylogeny. Based on these results the genus was found to be polyphyletic, with taxa tentatively identified as *Coleophoma* clustering in *Dothideomycetes* and *Leotiomycetes*. Species corresponding to the concept of *Coleophoma s.str.* (*Dermateaceae*, *Helotiales*, *Leotiomycetes*) were found to form a distinct clade, with five new species. Furthermore, *Coleophoma* was found to be linked to the newly established sexual genus, *Parafabraea*, which is reduced to synonymy. Isolates occurring on *Ilex aquifolium* in the Netherlands also clustered in *Dermateaceae*, representing a novel genus, *Davidhawksworthia*. In the *Dothideomycetes*, several taxa clustered in *Dothiora* (*Dothideaceae*, *Dothideales*), which is shown to have *Dothichiza* and *Hormonema*-like asexual morphs, with four new species. Furthermore, *Pseudocamaropycnis* is introduced as a new genus (*Mytilinidiaceae*, *Mytilinidiales*), along with *Briansuttonomyces* (*Didymellaceae*, *Pleosporales*) and *Dimorphosporicola* (*Pleosporaceae*, *Pleosporales*).

Introduction

The genus *Coleophoma* (von Höhnel 1907), typified by *Coleophoma crateriformis*, was established to accommodate coelomycetous fungi that are presently known to be plant pathogenic, saprobic or endophytic, occurring on a wide range of host plants. *Coleophoma* is characterised by having pycnidial conidiomata with well developed lower, but poorly developed upper walls, hyaline conidiophores intermingled among hyaline, collapsing paraphyses, and discrete, integrated phialidic conidiogenous cells with prominent periclinal thickening, and smooth, hyaline, cylindrical, guttulate, straight conidia with obtuse ends (Nag Raj 1978; Sutton 1980).

Species of *Coleophoma* differ in their ecology, being endophytic (e.g. *Coleophoma prunicola* in living leaves of *Prunus lusitanica*; Duan *et al.* 2007), saprobic (*Coleophoma empetri* on leaf litter; Wu *et al.* 1996), and plant pathogenic, e.g. *Coleophoma fusiformis* on leaves of *Rhododendron* (Sutton 1980; Duan *et al.*

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http://dx.doi.org/10.1016/j.funbio.2016.05.009

2007), *Coleophoma eucalypti* and *Coleophoma eucalyptorum* on *Eucalyptus* (Yuan 1996; Crous *et al.* 2011), *C. empetri* on *Vaccinium* (Polashock *et al.* 2009), *Coleophoma gevuinae* on *Gevuina* (Bianchinotti & Rajchenberg 2004), and *Coleophoma proteae* on *Protea caffra* (Crous *et al.* 2012).

Based on the phylogenetic position of *C. crateriformis*, De Gruyter *et al.* (2009) placed *Coleophoma* in *Dothideales*, while *Coleophoma maculans* grouped in *Helotiales*, showing the genus to be paraphyletic (Tanaka *et al.* 2015). In a subsequent study, Thambugala *et al.* (2014) confirmed *Coleophoma s.str.* to belong to *Dothideales* (*Dothideaceae*), being closely related to species of *Dothiora* and *Cylindroseptoria*. However, *Dothiora* is typified by *Dothiora pyrenophora*, which has *Dothichiza sorbi* as asexual morph (Sivanesan 1984). *Cylindroseptoria* is typified by *Cylindroseptoria ceratoniae*, but *Cylindroseptoria pistacina* was allocated to *Neocylindroseptoria* by Thambugala *et al.* (2014), as the genus was paraphyletic.

Several genera have to date been reduced to synonymy under *Coleophoma* based on morphology, namely *Basilocula*, *Ceuthosira*, and *Xenodomus* (Nag Raj 1978), as well as *Coleonaema*, *Bactropycnis*, and *Rhabdostromina* (Sutton 1980). Given differences in conidiomatal development between *Coleonaema* and *Coleophoma*, however, Duan *et al.* (2007) were of the opinion that *Coleonaema*, typified by *Coleonaema oleae*, should again be resurrected as distinct genus. Other than the few isolates included in phylogenetic studies dealing with other genera in *Dothideales*, the genus *Coleophoma*, which clearly includes several species associated with important plant diseases, remains insufficiently known, and in urgent need of revision (Sutton 1980). The aim of the present study was thus to employ morphology and multigene phylogenetic data to clarify relationships of *Coleophoma* among other genera in *Dothideaceae*, to resolve the paraphyletic nature of the genus, and also try to elucidate the host range of the various species known from culture.

Materials and methods

Isolates

The majority of the isolates used in this study were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. Isolates included were through the years identified as species of *Coleophoma* based on the fact that they had pycnidial conidiomata, and cylindrical conidia. In addition, fresh collections were made from conidiomata on symptomatic leaves of diverse hosts. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing pine needle agar (PNA) (Smith *et al.* 1996), 2 % malt extract agar (MEA), potato-dextrose agar (PDA), and oatmeal agar (OA) (Crous *et al.* 2009b), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation.

DNA isolation, amplification, sequencing, and phylogenetic analysis

Genomic DNA was isolated from fungal mycelium growing on MEA or OA, using the UltraClean™ Microbial DNA Kit (MO Bio, Carlsbad, CA, USA). The internal transcribed spacer region (ITS) was amplified with the primers ITS5 and ITS4 (White *et al.* 1990), or V9G (De Hoog & Gerrits van den Ende 1998), the large subunit of nrDNA (LSU) with LR0R (Vilgalys & Hester 1990) or LSU1Fd (Crous *et al.* 2009a) and LR5 (Vilgalys & Hester 1990), the β-tubulin gene (*tub2*) with T1 (O'Donnell & Cigelnik 1997) or Bt-2a and Bt-2b (Glass & Donaldson 1995), and translation elongation factor 1-alpha (*tef1*) with EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell *et al.* 1998) or EF1-986R (Carbone & Kohn 1999). PCR and reaction mixtures followed Groenewald *et al.* (2013) for ITS, *tef1*, and *tub2*, and Crous *et al.* (2009a) for LSU. PCR products were sequenced in both directions and a consensus sequence calculated, as described by Gomes *et al.* (2013).

Phylogenetic analyses

Novel sequences generated in this study were blasted against the NCBI's GenBank nucleotide database to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of different gene regions, including sequences obtained from this study and sequences downloaded from GenBank, were initially performed by using the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & Standley 2013), and then manually adjusted in MEGA v. 6.06 (Tamura *et al.* 2007). To check the congruence of different gene regions, individual gene trees were manually compared prior to concatenation. Maximum parsimony (MP; LSU overview and species phylogenies) and Bayesian analyses (LSU overview phylogenies) were used to determine the phylogenies. The MP analyses were conduct in PAUP v. 4.0b10 (Swofford 2003) with the heuristic search option set to 100 random taxa addition, and the tree bisection-reconnection (TBR) as the branch-swapping algorithm. All characters were weighted equally and alignment gaps were treated as new state data and bootstrap analyses were based on 1000 replications. Tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) values were also calculated. Bayesian analyses were performed in MrBayes v. 3.2.5 (Ronquist *et al.* 2012) and the best nucleotide substitution model per gene region was selected using MrModeltest v. 2.3 (Nylander 2004). The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The heating parameter was set to 0.2 and trees were sampled every 100 generations. Analyses stopped once the average standard deviation of split frequencies was below 0.01. Sequences generated in this study were deposited in GenBank (Table 1) and alignments and phylogenetic trees in TreeBASE (www.treebase.org). Nomenclatural novelties were deposited in MycoBank (Crous *et al.* 2004).

Morphology

Observations were made with a Nikon SMZ25 stereomicroscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and a Nikon DS-Ri2 camera and software. Colony characters and pigment production were noted after 2 wk of growth on MEA, PDA, and OA incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Morphological descriptions were based on