Colletotrichum: a catalogue of confusion

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Hyde, K.D., Cai, L., McKenzie, E.H.C., Yang, Y.L., Zhang, J.Z. and Prihastuti, H. (2009). *Colletotrichum*: a catalogue of confusion. Fungal Diversity 39: 1-17.

Identification of *Colletotrichum* species has long been difficult due to limited morphological characters. Single gene phylogenetic analyses have also not proved to be very successful in delineating species. This may be partly due to the high level of erroneous names in GenBank. In this paper we review the problems associated with taxonomy of *Colletotrichum* and difficulties in identifying taxa to species. We advocate epitypification and use of multi-locus phylogeny to delimit species and gain a better understanding of the genus. We review the lifestyles of *Colletotrichum* species, which may occur as epiphytes, endophytes, saprobes and pathogens. It is not clear in most cases whether taxa isolated from these different life modes are the same species, or different morphologically similar species; in most cases identification has been based on morphology and may not be accurate. We use three selected species, *C. dematium*, *C. destructivum* and *C. fragariae* to highlight the problems associated with species identification and the problems that may occur when wrong names are applied to species. We also review clinical aspects of the genus and the use of *Colletotrichum* species in biotransformations. In most examples, the need for correct identification, which can be achieved by contrasting with types and comparison of molecular data, is stressed. We propose the need for agreement on protocols to deal with description and naming of *Colletotrichum* species and make predictions for the next five years. The reviews serve to illustrate the importance of correctly identifying strains before commencement of scholarly research.

Key words: biocontrol, biotransformation, mycoses, novel compound discovery

Article Information
Received 27 October 2009
Accepted 2 December 2009
Published online 9 December 2009
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Introduction

What we now know as *Colletotrichum* was first reported by Tode (1790) in the genus *Vermicularia*. *Colletotrichum* itself was introduced by Corda (1831) and is now known to comprise "coelomycetes" with a *Glomerella* teleomorph stage (Sutton, 1992; Shenoy *et al*., 2007a; Hyde *et al*., 2009). *Colletotrichum* encompasses species with endophytic, epiphytic, saprobic and phytopathogenic lifestyles (Kumar and Hyde, 2004; Photita *et al*., 2001a,b, 2003, 2004; Liu *et al*., 2007; Prihastuti *et al*., 2009), as well as human pathogens (Cano *et al*., 2004). The genus has worldwide importance, causing diseases on a wide range of economic crops and ornamental plants (Sutton, 1992; Than *et al*., 2008a-c; Hyde *et al*., 2009). *Colletotrichum* has continued to rank highly as one of the most studied genera of phytopathogenic fungi. Latunde-Dada (2001) concluded that *Colletotrichum*, as judged by the number of hits recorded in the *Web of Science* over the period 1981 to March 2001 rated with *Botrytis*,

Puccinia and *Verticillium*. *Colletotrichum* is only surpassed by *Fusarium, Phytophthora* and *Rhizoctonia* in phytopathogenic publications.

The objective of this paper on *Colletotrichum* is to 1) review the problems in taxonomy and difficulties of identifying taxa to species, 2) review the relationships and lifestyles, 3) review clinical aspects and 4) review industrial use (e.g. biotransformations). In all cases we discuss the potential problems with misidentification of taxa. Extensive research by numerous researchers has been carried out on *Colletotrichum dematium*, *C. destructivum* and *C. fragariae* and these data are reviewed. The reviews serve to illustrate the importance of correctly identifying strains before commencement of scholarly research.

Taxonomic systematics of *Colletotrichum* species

Identification within the genus *Colletotrichum* is complicated as species have few distinguishing morphological characters, and because teleomorphic stages are rarely formed (Hyde *et al*., 2009). Some taxa have uncertain or extensive host relationships and pathological variations, and are often morphologically variable in culture (Simmonds, 1965; Bailey and Jeger, 1992; TeBeest *et al*., 1997; Freeman *et al*., 2000; Latunde-Dada, 2001; Du *et al*., 2005; Thaung, 2008). TeBeest *et al*. (1997) concluded that taxonomic uncertainty has made accurate identification difficult and complicated efforts to understand host relationships, diagnose diseases accurately, develop effective control strategies and establish cost effective quarantine programs.

Traditionally, *Colletotrichum* species have been identified and delimited on morphological characters; several features have been utilized by taxonomists including size and shape of conidia and appressoria; presence or absence of setae, sclerotia, acervuli and teleomorph state and cultural characters such as colony colour, growth rate and texture (Simmonds, 1965; Smith and Black, 1990; Sutton, 1992; TeBeest *et al*., 1997; Photita *et al*., 2005; Than *et al*., 2008a-c; Thaung, 2008). These criteria alone are not always adequate for reliable differentiation among *Colletotrichum* species due to variation in morphology and phenotype among species under environmental influences. To overcome the inadequacies of these traditional schemes, molecular techniques have been used to characterize and identify taxa within *Colletotrichum* (Sreenivasaprasad *et al*., 1996; Abang *et al*., 2002; Moriwaki *et al*., 2002; Peres *et al*., 2002; Guerber *et al*., 2003; Photita *et al*., 2005; Du *et al*., 2005; Shenoy *et al*., 2007b; Whitelaw-Weckert *et al*., 2007; Peres *et al*., 2008; Than *et al*., 2008a-c). Cannon *et al.* (2000) stated that nucleic acid analyses should provide the most reliable framework to classify *Colletotrichum*, as DNA characters were not directly influenced by environmental factors.

The combined use of molecular diagnostic tools along with traditional morphological techniques is at present an appropriate and good approach for studying *Colletotrichum* species complexes (Cannon *et al*., 2000; Cai *et al*., 2009). Photita *et al*. (2005) separated 34 isolates of *Colletotrichum* isolated from seven hosts in Thailand into five morpho-groups viz: *C. musae*, *C. gloeosporioides* group 1, *C. gloeosporioides* group 2, *C. gloeosporioides* group 3 and *C. truncatum*. Whitelaw-Weckert *et al.* (2007) proposed a new *C. acutatum* group, which is now included in *C. simmondsii* (Shivas and Tan, 2009), based on cultural, morphological, RAPD-PCR and sequencing of parts of the rDNA-ITS regions and the β-tubulin gene. Than *et al*. (2008b) differentiated the isolates of chilli anthracnose from Thailand into three species viz: *C. acutatum*, *C. capsici* and *C. gloeosporioides* (but see page 3) based on morphological characterization, sequencing based on rDNA-ITS and β-tubulin gene and pathogenicity testing. Thus, accurate identification of *Colletotrichum* species can be achieved by combining multigene analysis and morphological characters. Once a species is accurately named, it unlocks data that can be used for developing and implementing effective disease control strategies (Freeman *et al*., 1993) and other research.

One of the problems with accurate identification of *Colletotrichum* species using multigene analysis or barcoding is that many existing sequences in GenBank are basically wrongly named. Crouch *et al*. (2009a) have revealed a high frequency of misidentification (86%) based on rDNA-ITS sequence similarity comparison in GenBank within the *C. grami-*

nicola species complex. Cai *et al*. (2009) analyzed the 343 rDNA-ITS sequences of *C. gloeosporioides* in GenBank (accessed 06-Sept-2009) and found that an astounding 86% of these sequences show considerable evolutionary divergence from the epitype of *C. gloeosporioides* and most likely represent other species. These are remarkably high error rates and show that accurate identification of *Colletotrichum* species using rDNA-ITS sequence data presently lodged in GenBank is impossible. How can this situation be rectified? Isolates that represent the types of species are needed. Published new species must have ex-type living strains deposited in easily accessible culture collections for future work. In some cases (e.g. *C. boninense*, *C. kahawae*) the living type is available, however in most cases it is not. Epitypification is necessary in certain cases, such as where the type specimen of the taxon no longer exists, or is in poor condition, or of ambiguous status, or has deteriorated so that many important features are not available for further studies (Shenoy *et al*., 2007b; Cannon *et al*., 2008; Hyde and Zhang, 2008; Than *et al*., 2008a). Epitypification can solve many taxonomic problems and stabilize the understanding of species, genera, families or orders (Phillips *et al*., 2007; Shenoy *et al*., 2007b; Than *et al*., 2008a; Cannon *et al*., 2008; Hyde and Zhang, 2008). Epitypification of *Colletotrichum* species commenced in 2007 and now 42 currently used species have been epitypified or have living cultures (Hyde *et al*., 2009).

Colletotrichum lifestyles

Colletotrichum species have been reported to occur as endophytes, epiphytes, saprobes, plant pathogens and even human pathogens (Sutton, 1992; TeBeest *et al*., 1997; Cano *et al*., 2004; Kumar and Hyde, 2004; Photita *et al*., 2004, 2005; Promputtha *et al*., 2007). *Colletotrichum* species that cause serious plant disease are also commonly isolated as endophytes from healthy plants and have been identified as saprobes on dead plant material (Photita *et al*., 2001a, 2003; Kumar and Hyde, 2004; Liu *et al*., 2007; Promputtha *et al*., 2007; Damm *et al*., 2009; Prihastuti *et al*., 2009).

In many cases the same species have been recorded with several lifestyles although with the ambiguity of species identification it is not always clear whether they are definitely the same species. How and if taxa change their lifestyle from non-pathogenic to pathogenic still needs to be addressed and is an important unanswered question in the study of *Colletotrichum*. Full descriptions of the life styles for each *Colletotrichum* species linked with molecular data for accurate taxon identification are needed to explore, understand and develop effective control strategies in the genus. In one such study, Prihastuti *et al*. (2009) described *C. fructicola* and *C. siamense* from coffee berries, isolated as epiphytes, endophytes and pathogens and these species have since been shown to be widespread on several hosts (Yang *et al*., 2009). *Colletotrichum dematium* also occurs as an endophyte, pathogen and saprobe (Damm *et al*., 2009). The significance of these findings may have great importance as evidence grows that these species of *Colletotrichum* are ubiquitous and widespread.

Colletotrichum as plant pathogens and agents of post harvest disease

Colletotrichum is one of the most economically important genera of fungi, causing anthracnose disease, affecting a wide host range, especially on tropical and subtropical crops as well as fruit trees (Sutton, 1992). Above ground plant parts can be affected by *Colletotrichum* diseases at all stages on stems, leaves, flowers and fruits. An example of *Colletotrichum* anthracnose familiar to many is the blackening of tropical fruits (Tang *et al*., 2005), especially bananas and mangoes in fruit bowls.

Crouch and Beirn (2009) review the graminicolous species of *Colletotrichum* in this issue, while Hyde *et al*. (2009) discuss diseases caused by *Colletotrichum* species, whose names are in current use. The disease often takes two forms, resulting in spots on leaves or the blackening of fruits, usually post-harvest. In the case of persimmon, *Colletotrichum horii* can infect fruits, twigs, cause dieback, and even tree death (Zhang, 2008). *Colletotrichum acutatum, C. capsici* and *C. gloeosporioides* have been reported causing anthracnose disease

on chilli fruits in Thailand (Than *et al*., 2008b). However, the application of these three species names needs care as *C. acutatum sensu lato* includes three recently named species (Shivas and Tan, 2009); *C. capsici* is now known as *C dematium*; and *C. gloeosporioides* from chilli is either *C. asianum* or *C. fructicola* (Damm *et al*., 2009; Cai pers. comm.). Because of their importance in phytopathology, plant breeding and biosecurity, species need to be correctly identified and this has not previously been easy due to the lack of taxonomically informative characters. Much has been written on *Colletotrichum* as plant pathogens, albeit in term of species *sensu lato* (e.g. Sutton, 1980, 1992) and interested readers should refer to these texts for further data. Three examples are also discussed later in the paper.

Colletotrichum as endophytes

Colletotrichum species have been found as symptomless inhabitants (endophytes) in plant tissues (Liu *et al*., 2007; Damm *et al*., 2009; Prihastuti *et al*., 2009). For example, putative *C. gloeosporioides* and *C. acutatum* strains were isolated from healthy leaves and pseudostems of banana (*Musa acuminata*), ginger (*Alpinia malaccensis*), *Euphatorium thymifolia* and wild ginger (*Amomum siamense*) in Thailand, and in low frequencies from rhizomes of wild ginger (Bussaban *et al*., 2001; Photita *et al*., 2005). Lu *et al*. (2004) isolated *C. gloeosporioides* and *C. boninense* as endophytes from leaves of 12 different tree species in the Iwokrama Forest Reserve, Guyana. Hyde and Soytong (2008) discussed the role of endophytes that can become primary saprobic decomposers or as latent infections of pathogens that cause disease under specific conditions. Simmonds (1941) showed in field experiments that *Gloeosporium musarum* (= *C. musae*) can remain latent within the skin of green banana fruits for almost five months, and develop anthracnose fruit rot as the fruit ripens. The development of the fungus in the latent phase is restricted due to a poor capacity for secreting macerating enzymes and pectinesterase (Simmonds, 1963). Quiescent infections of strawberry transplants by *C. gloeosporioides* are common and play an important role as inoculum sources of anthracnose crown rot (Raman and Louws, 2008).

Wright (1998) studied *Colletotrichum* infection of *Citrus* and isolated *Colletotrichum* as endophytes from growing stems over an entire season. She consistently isolated *C. gloeosporioides*, which appeared to grow into new stem tissues and eventually into the flower and infected flowers at fruit set (Wright *et al*., 1996, 1997; Wright, 1998). Pathogenicity testing also showed that the endophytic strains of *C. gloeosporioides* caused disease of oranges and Wright (1998) speculated that the endophytes grow into the albedo of oranges and remain dormant until a period of post harvest storage somehow triggers the fungus to become active and cause stem end fruit rot (Wright *et al*., 1996, 1997). The strain was not compared with the epitype, which was not available at the time, but this is likely to be *C. gloeosporioides sensu stricto* as the epitype is from oranges.

Colletotrichum as saprobes

Colletotrichum species are rarely recorded in studies concerning saprobes on various hosts (e.g. pine needles, Zamora *et al*., 2008; *Castanopsis diversifolia* leaves, Duong *et al*., 2008; *Magnoliaceae* wood, Kodsueb *et al*., 2008), although Photita *et al*. (2001a, 2003) have recorded *C. gloeosporioides* as a saprobe on banana (*Musa* sp.) and Osono *et al*. (2009) recorded *C. gloeosporioides* on decaying leaves of *Shorea obtusa*. *Colletotrichum gloeosporioides* and a *Colletotrichum* species have been also recorded on dead leaves of *Draceana lourieri* (Thongkantha *et al*., 2008). It appears that *Colletotrichum* species survive and compete poorly as saprotrophs. In the *Compendium of Soil Fungi*, Domsch *et al*. (2007) listed only two species; *C. dematium* as a common saprotroph on dead plant material, and *C. gloeosporioides* that has rarely been reported from soil. Conidia of *C. acutatum* and C. *gloeosporioides* isolates from strawberry survive for up to 1 year in autoclaved soil, whereas the viability declined rapidly within a few days in untreated soils at 22% soil moisture (field capacity) (Freeman *et al*., 2002). The number of conidia of *C. gloeosporioides*, causal agent of water yam (*Dioscorea alata*) anthracnose in Guadeloupe, was higher in artificially inoculated residues on the soil surface than in residues buried at 0.1 m soil

depth, which decomposed faster (Ripoche *et al*., 2008). In Finland, *C. acutatum* can survive one winter in strawberry residues on the soil surface or covered with soil and in infected weed debris and infect young strawberry plants in greenhouse tests (Parikka *et al*., 2006).

In most of the above studies the species were identified based on morphology and not compared with types or at the molecular level and thus the names of species are most likely wrong. Future studies must be compared against the types using molecular data.

Problems associated with selected species

Much has been written about many individual species of *Colletotrichum* and yet in most cases the name used represented a species complex and it is unclear which actual species is involved. In the following section we review three species of *Colletotrichum* in order to highlight the problems associated with inadequate knowledge of the species and possible incorrect or inaccurate naming.

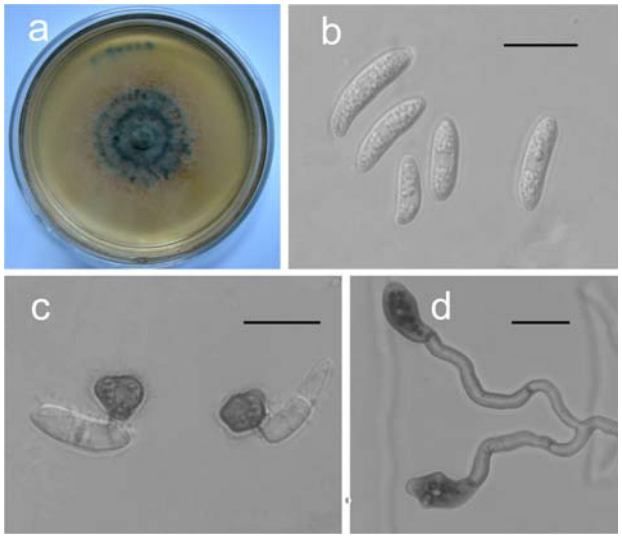
Colletotrichum dematium (Pers.) Grove

Colletotrichum dematium until recently was a relatively poorly known species in urgent need of epitypification. It was originally collected from a stem of *Eryngium* in France as well as solanaceous hosts (see Damm *et al*., 2009 for detailed discussion) and has been more recently recorded from numerous hosts such as a pathogen of chilli (Than *et al*., 2008c). It has been also recorded as a pathogen of *Polygonatum falcatum* (Tomioka *et al*., 2008) and an endophyte of *Pteromiscum* sp. (Ren *et al*., 2008). Disease symptoms are reported to range from fruit rot to shoot, leaf, and flower blight, e.g., Sutton (1980) reported that in herb. IMI it was represented by 216 collections from 37 countries on 118 different host genera. Many putative hosts are commercial plants, such as tomato (Bello, 2000), mulberry (Yoshida *et al*. 2002), soybean (Fakir, 1979; Shovan *et al*., 2008) and beech (Sahashi *et al*., 1995). The symptoms on mulberry leaf are brown necrotic spots or streaks (Yoshida and Shirata, 1998). Tomato anthracnose results in greyish, sunken, water-soaked lesions on tomato fruits and later the centre of the spots become tan and flecked with small black specks, and black acervuli form in concentric rings (Bello, 2000). On beech, *C. dematium* was reported to cause post-emergence damping-off of current-year beech seedlings (Sahashi *et al*., 1995).

The pathogenicity of *C. dematium* on mulberry, beech seedlings and *Polygonatum falcatum* has also been tested (Sahashi *et al*., 1995; Yoshida and Shirata, 1998; Yoshida *et al*., 2002; Tomioka *et al*., 2008). These studies proved that putative strains of *C. dematium* are pathogenic to various hosts. Yang *et al*. (2009) also isolated this taxon from amaryllids with anthracnose symptoms.

Colletotrichum dematium is difficult to recognize based on morphological characteristics, mainly because different researchers have described conidia width differently (http://www.mycobank.org/BioloMICSServer.aspx?Link=T&Rec=120313). Colonies of putative *C. dematium* strains have been reported by Sutton (1992) to be very variable with white to pale mouse-grey or grey-vinaceous patches with abundant setae and black, conical sclerotia. Conidia are formed in olive-grey to light vinaceous-salmon masses, and are 18–26 × 2–3 µm, falcate, fusiform, and gradually tapered to each end (Sutton, 1992). Appressoria are medium brown, clavate, ovate to irregular, margin entire or slightly irregularly lobed (Sutton, 1992). Bobev *et al*. (2009) reported *C. dematium* (spores mean sizes: 22 × 4.5 µm, ranging from 18.3–25 × 4.2–5.8 µm, and 99% similar to an isolate of *C. dematium* (GenBank Accession No. AJ301954; strain BBA 62147) infecting *Goniolimon tataricum*. In Yang *et al.* (2009) study on amaryllids, putative *C. dematium* spores were 13.5–19 × 2.5–4 µm ($\bar{x}$ = 15.8 × 3.2 µm, n = 50). When combined with other morphological and molecular characteristics we defined our species as *C. dematium* (Fig. 1), but since the species was presently not epitypified we could not be definite about this name. This taxon therefore urgently needed epitypifying so that researchers could obtain accurate reference criteria, as is now available for *C. gloeosporioides* (Cannon *et al*., 2008).

Damm *et al*. (2009) epitypify *C. dematium* in this issue, with conidial sizes of (18–)20–23(–24) × 3–4(–5.5) µm. The species in Yang *et al*. (2009) was shown to be different



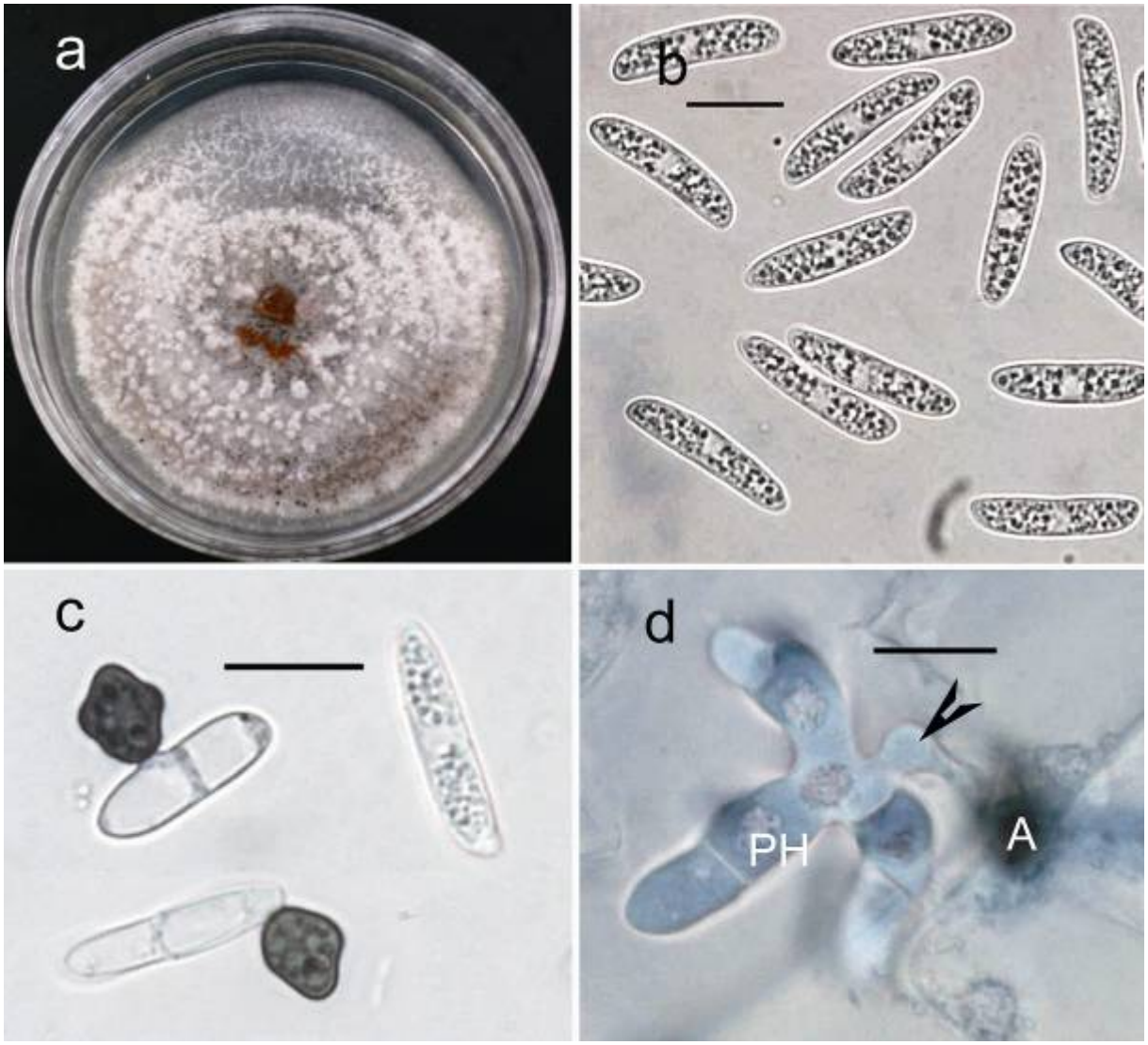
Figs 1a-d. Putative strain of *Colletotrichum dematium* (later shown to be *C. spaethianum*): **a.** Colony on PDA at 25ºC, 7 days. **b.** Conidia on PDA. **c.** Appressoria. **d.** Hyphopodia. Bars = 10 µm.

and is named *C. spaethianum*. This is an excellent example of the importance of epitypification and how it helps stabilize names and allows comparison in various studies on *Colletotrichum* species. Only a small number of strains were included in the species concept and many strains previously reported as *C. dematium sensu lato* were shown to represent other species, i.e. *C. circinans, C. dematium, C. lilii, C. lineola, C. liriopes, C. spaethianum, C. spinaciae, C. tofieldiae, C. trichellum, C. truncatum* and two unidentified species. The outcome has enormous phytopathological significance. Even though a small number of strains were included as *C. dematium sensu stricto*, these strains indicate that it has a wide host range and occurs as an endophyte, pathogen and saprobe (Damm *et al*., 2009).

Colletotrichum destructivum O'Gara

Colletotrichum destructivum was described from red clover (*Fabaceae*) by O'Gara (1915) and has been confused with several species including *C. gloeosporioides*, *C. lindemuthianum* and *C. truncatum*. In reviewing the genus, von Arx (1957) maintained *C. destructivum* as a distinct species. *Colletotrichum destructivum* was also accepted in von Arx (1970). Sutton (1980) did not consider *C. destructivum* an acceptable species; he probably thought of it as a synonym of *Glomerella cingulata*. Manandahar *et al.* (1986) provided a detailed description of the morphology and pathogenicity of *G. glycines* and first established the connection between *C. destructivum* and *G. glycines*. Appressoria size and conidia length in *C. destructivum* is considered to be similar to that in *C. lindemuthianum*. However, *C. destructivum* and *C. lindemuthianum* have been differentiated by cultural characteristics, conidia width, and their respective teleomorphs (Manandahar *et al.*, 1986). More recently, due to the similarity of morphology of conidia and appressoria, the infection process and the rDNA-ITS sequence data, *C. higginsianum* was considered to be a synonym of *C. destructivum* (O'Connell *et al*., 2004). Sun and Zhang (2009) isolated putative *C. destructivum* strains from cowpea and found that their morphological characters (e.g. colony, conidia and appressoria) (Figs 2a-c) were similar to *C. destructivum* (*sensu* Sutton, 1992) and their infection process and intracellular infection structures (Fig. 2d) on cowpea also were consistent with that of *C. destructivum* on other hosts (Bailey *et al*., 1990; Latunde-Dada *et al*., 1997; Shen *et al.*, 2001). However, cowpea isolates with rDNA-ITS sequences identical to that of *C. higginsianum* from cruciferous hosts could infect and complete the asexual cycle on *Arabidopsis thaliana*. The infection process showed that in the initial biotrophic phase, intracellular primary hyphae were confined to one epidermal cell, whereas in the subsequent necrotrophic phase, secondary hyphae invaded the neighboring cells, in the same way as *C. higginsianum* originating from cruciferous plants (O'Connell *et al*. 2004). This implied that the host range or the cruciferous hosts are not reliable criteria to delimit the two species. Thus O'Connell *et al*. (2004) proposed *C. higginsianum* to be a synonym of *C. destructivum*.

Latunde-Dada and Lucas (2007) showed that the nucleotide sequences of the D2 and ITS-2 regions of rDNA amongst *C. truncatum*, *C. destructivum* and *C. linicola* had very high similarities (97-99%), and proposed, by a combination of phylogenetic relationships, as well as morphology, infection processes and intracellular infection structures that *C. destructivum* was a species aggregate, which also includes *C. linicola* and *C. truncatum*. The



Figs 2a-d. Morphological characteristics of colony, conidia, appressoria and infection structures of a putative strain of *Colletotrichum destructivum* from cowpea (*Vigna unguiculata*). **a.** Colony on PDA. **b.** Conidia from PDA. **c.** Appressoria produced from the germinated conidia on hydrophobic polystyrene. **d.** Infection vesicle (arrow) and primary hyphae (PH) in tissue of *V. unguiculata* cv. qiudou 512 72 hours after inoculation. Note that an appressorium (A) produces an infection peg which invades a host epidermal cell to form a globose infection vesicle (arrowed), and developing branched primary hyphae (PH). Bars = 10 μm

above discussion illustrates the confusion surrounding the status of *C. destructivum* and closely related species which is still unresolved.

Many C*olletotrichum* species have been described on legumes, mostly from crops in temperate regions. *Colletotrichum destructivum* is capable of causing anthracnose disease in lucerne (Boland and Brochu, 1989). It has been reported to cause considerable yield losses in Europe (Pauly, 1974; Robotic and Klokocar-Smit, 1983), North America (Boland and Brochu, 1989), northern Africa (Troeung and Gosset, 1987) and South Africa (Thompson and van der Westhuizen, 1985; Koch *et al*., 1989). However, in a complex infection with *C. coccodes* (Wallr.) Hughes, *C. dematium* (Pers.) Grove, *C. truncatum* (Schwein.) Andrus & Moore and *C. trifolii* Bain, it is considered as a secondary pathogen (Graham *et al*., 1976; Koch *et al*., 1989). The host range of *C. destructivum* is wide and includes legumes such as *Glycine max*, *Leucaena leucocephala*, *Lotus* spp., *Melilotus albus*, *Phaseolus lathyroides*, *Trifolium* spp., *Vigna unguiculata*, *Coronilla varia*, as well as tobacco (*Nicotiana tabacum*), dodder (*Cuscuta* spp.) and *Arabidopsis thaliana* (Forer *et al*., 1973; Massenot and Raynal, 1973; Baxter *et al*., 1983; Manandahar *et al*., 1986; Wolcan and Bello, 1988; Koch *et al*., 1989; Latunde-Dada *et al*.,

1996; O'Connell *et al*., 2004), although these records need confirmation with sequence data. Host-specificity has also been observed among *C. destructivum* isolates (Wolcan and Bello, 1988).

The infection processes and intracellular infection structures of strains named *C. destructivum* is well understood (Fig. 2d), with similar structures in cowpea (Bailey *et al*., 1990; Latunde-Dada *et al*., 1996), alfalfa (Latunde-Dada *et al*., 1997) and tobacco (Shen *et al.* 2001). *C. truncatum* and *C. linicola* (Latunde-Dada and Lucas, 2007), and *C. higginsianum* have similar infection processes and intracellular infection structures (O'Connell *et al*., 2004).

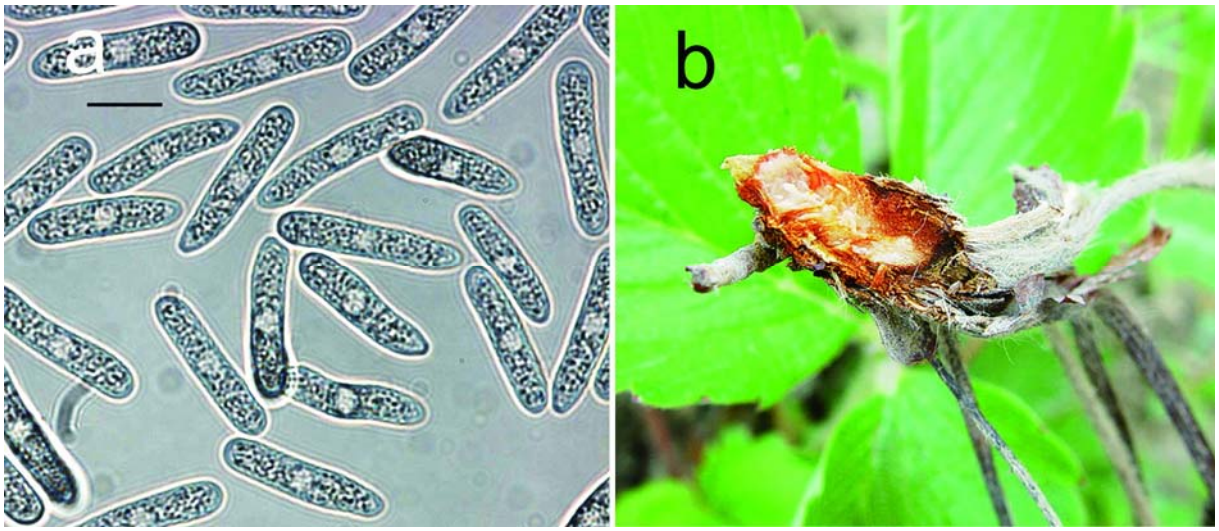
Much of the above confusion is based on the fact that *C. destructivum* has not been epitypified and that types are not available for sequence data comparison. Thus epitypification is urgently required and then species named as "*destructivum*" can be verified against the epitype. Similarly, it will be possible to establish whether the strains from cowpea in the infection process studies are correctly named.

Colletotrichum fragariae Brooks

Strawberry (*Fragaria ananassa*) anthracnose is caused by three putative *Colletotrichum* species: *C. acutatum, C. fragariae* and *C. gloeosporioides* (Howard and Albregts, 1983; Smith and Black 1986). *Colletotrichum acutatum* and *C. gloeosporioides* are reported to have a wide host range, while *C. fragariae* is restricted to strawberry plants (Gunnell and Gubler, 1992). These species have often been confused because each produces similar symptoms on strawberry including crown rot (Fig 3b), fruit rot and lesions. *Colletotrichum fragariae* was described from Florida by Brooks (1931) and causes a very destructive crown rot (Howard and Albregts, 1983). However, it is poorly defined and preliminary studies of isolates from strawberry showed that the criteria used in species identification are not reliable (Smith and Black, 1990). Some isolates identified as *C. fragariae* because they lacked a teleomorph conformed to *C. gloeosporioides*. Other isolates, classified as *C. fragariae* or *C. gloeosporioides* that produced a red pigment in culture corresponded to *C. acutatum* (Gunnell and Gubler, 1992). Since host origin was deemed less important, von Arx (1957) placed *C. fragariae* in synonymy with *C. gloeosporioides*, but researchers have generally retained the use of the name *C. fragariae* when dealing with the pathogen on strawberries (Howard and Albregts, 1984; Mass and Howard, 1985; Sutton, 1992).

Gunnell and Gubler (1992) reported that *C. fragariae* could be clearly distinguished from *C. acutatum* and *C. gloeosporioides* by the morphology on strawberry leaf agar. Delimitation of these species remains problematic because literature references to *C. acutatum* and *C. gloeosporioides* may actually refer to other species (Shivas and Tan, 2009; Sreenivasaprasad *et al*., 1994).

Several molecular studies have attempted to resolve relationships of *C. fragariae* and other species from strawberry, but studies to date are inconclusive. The techniques include analysis of zymograms (Bonde *et al*., 1991), mitochondrial DNA restriction fragment length polymorphisms (mtDNA RFLPs) (Sreenivasaprasad *et al.*, 1992; Freeman *et al.*, 1993), arbitrarily primed polymerase chain reactions (AP-PCR) (Freeman and Rodriguez, 1995; Freeman and Katan, 1997), random amplified polymorphic DNA (RAPD) (Martínez-Culebra *et al*., 2002) and ribosomal DNA (rDNA) restriction analyses (Sreenivasaprasad *et al*., 1992, 1994). Although molecular studies have led to the establishment of different molecular groups within *Colletotrichum* isolates from strawberry, the assignment of taxonomic ranks is difficult, because these techniques were developed for characterization. rDNA sequence analysis is a useful tool for species delimitation in *Colletotrichum* (Sherriff *et al*., 1994, 1995; Sreenivasaprasad *et al*., 1993, 1994), but multigene loci analysis has proved more definitive. The divergences of rDNA-ITS sequences between isolates of *C. fragariae* and *C. gloeosporioides* is too low to confidently separate the species (Sherrif *et al*. 1994; Sreenivasaprasad *et al*., 1996; Martínez-Culebras *et al*., 2003) and it was suggested that *C. fragariae* falls within the *C. gloeosporioides sensu lato* (Sreenivasaprasad *et al*., 1996). Martínez-Culebras *et al*. (2000) found that there was only a *MvnI* specific site among ITS1 region of the isolates of *C. gloeosporioides*



Figs 3a, b. Conidia of a putative strain of *Colletotrichum fragariae* and its crown rot symptom on strawberry. **A.** Cylindrical conidia with one end rounded and the other pointed (grown on strawberry leaf agar). **B.** Internal brown necrotic lesions of crown rot caused by *C. fragariae*. Bar = 10 μm.

instead of *C. fragariae*. This trait was confirmed subsequently by different original sequence data and could be used for differentiating *C. gloeosporioides* from *C. fragariae* (Martínez-Culebras *et al*., 2003), but it was considered that more isolates of *C. fragariae* and *C. gloeosporioides* from straw-berry and also *C. gloeosporioides* from different hosts should be studied. Recent phylogenetic research using a wider range of genes has been used to study the *C gloeosporioides* aggregate from strawberry (Johnston, in litt.) and supports the work of MacKenzie *et al*. (2007) indicating that *C. fragariae* is distinguishable from the rest of the *C. gloeosporioides* aggregate, but that it is not restricted to strawberry.

The drawback in most of the above studies is that the strains used were not compared with the type species. Recently *C. gloeosporioides* has been epitypified and multigene loci have been sequenced for this epitype. It is now possible to establish if the strains from strawberry are really *C. acutatum*, *C. gloeosporioides* or one of the recently described species, e.g. *C. simmondsii.* Whether *C. fragariae* (which requires epitypification) is a distinct species still needs resolving.

Colletotrichum in human disease

Although *Colletotrichum* species are mainly responsible for plant disease, several species have been reported to cause human disease as opportunistic pathogens (Table 1). Cano *et al*. (2004) listed five species of clinical importance while Damm *et al*. (2009) confirmed, using molecular data, that *Colletotrichum truncatum* was isolated from a corneal ulcer of a human eye. *Colletotrichum dematium* was also reported to cause eye keratitis (Giaconi *et al*., 2006) and fungal endophthalmitis, a potential devastating ocular disease that causes poor visual outcome (Chakrabarti *et al*., 2008). Cano *et al*. (2004) considered that it was important to find a quick unambiguous molecular test to distinguish *Colletotrichum* isolates, as prompt diagnosis was necessary as some drugs were not active against certain *Colletotrichum* species. For instance, itraconazole was not active *in vitro* against *C. coccodes* and *C. dematium*, but was active against some isolates of *C. gloeosporioides*. Cano *et al*. (2004) provided a table of morphological characters and also conducted a sequence analysis based on ribosomal DNA (rDNA) dataset to differentiate their clinical strains. Their phylogenetic analysis employed to separate the species was rather simple being based on a very short fragments of rDNA-ITS sequence (ranging from 124-174 bp) and not compared to the ex-type strains. Although we have tried to integrate these short sequences into the rDNA-ITS dataset of Cai *et al*. (2009), their phylogenetic positions could not be conclusively determined (L. Cai, pers. comm.).

Table 1. Clinical *Colletotrichum* species.

Taxon	Disease	Verified using molecular data	Isolate location	Reference
C. coccodes		No		Cano *et al*., 2004
C. crassipes		No	CBS 109355	Cano *et al*., 2004
C. dematium	Keratitis	No		Cano *et al*., 2004; Giaconi *et al*., 2006; Chakrabarti *et al*., 2008
C. gloeosporioides		No	CBS 102275	Cano *et al*., 2004
C. graminicola		No		Cano *et al*., 2004
C. truncatum	Isolated from corneal ulcer of human eye	Yes	IMI 266002	Damm *et al*., 2009

Cano *et al*. (2004) used two strains representing two species with clinical origin and compared these against 18 non-clinical strains. Some of the strains have since been shown to be wrongly named (e.g. *C. dematium* (*C. truncatum*) CBS 351.73 has been shown to be *C. circinans,* while *C. dematium* CBS 167.49 is *C. spaethianum*). It is not clear if the other non-clinical stains had been correctly named and thus it is not clear if the correct names were applied to the clinical strains. With the recent epitypification of *C. dematium*, *C. gloeosporioides* and *C. graminicola* it is now possible to establish if these clinically important strains were correctly named. There is an urgent need for mycologists to revisit the clinical *Colletotrichum* species following recent epitypification to establish which species are involved in human disease.

Colletotrichum in biotransformation

The use of *Colletotrichum* species in biotransformation was reviewed by García-Pajón *et al*. (2003) and there have been some publications since (e.g. Bastos *et al*., 2007; Bajpai *et al*., 2009). Several species have been studied for use in biotransformation, with putative strains of *C. gloeosporioides* having received most attention (García-Pajón *et al*., 2003). The importance and potential use of the genus in industrial applications has received more attention in the last 15 years.

Biotranformations using *Colletotrichum* species have included detoxification of phytolectins, biotransformations of saturated and unsaturated acyclic terpenoids, monoterpenoids and related cyclic compounds including ketone, cyclic monoterpenes, sesquiterpenes and steroids (Table 2). In these studies the *Colletotrichum* strains used have been given specific names, but it is unclear whether these names are correct. For example in Table 2, the strain of *C. acutatum* could be one of three species presently known from the species complex (Shivas and Tan, 2009); the strains of *C. atramentarium* and *C. lini* used do not represent currently applied names (see Hyde *et al*., 2009); the strains of *C. capsici* and *C. dematium* f. *truncatum* may be the one species, as they are now considered to be synonyms of *C. truncatum* (Damm *et al*., 2009); and the strains of *C. gloeosporioides* (and *G. cingulata*) may represent several different species within this species complex (Hyde *et al*., 2009). It is essential to apply correct names to strains used in biotechnology, since results of these studies may need to be verified, repeated or compared, and patents may be forthcoming. In all cases it would be problematical if taxa were wrongly named.

Future studies

A review of *Colletotrichum* indicates that species may have been wrongly named in many studies on biotransformation, clinical, pathogen, endophyte, molecular, saprobe and other aspects. It is essential that correct names are used in future so that comparisons between studies can be confidently made. It is presently impossible to compare species used in many studies as a name given by one group of researchers may be different to that given by other researchers, even though they are working with the same species. Similarly, the same name may be given to different species. Furthermore, the name of species with gene sequences deposited in GenBank must be verified before deposition, so that GenBank no

Table 2. Selected examples of species of *Colletotrichum* used in biotransformations.

Taxon	Biotransformation reaction	Reference
C. acutatum	Transformation of 2-phenylethanol and acetophenone	Aristizábal *et al*., 2008
C. atramentarium	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. capsici	Conversion of docosahexaenoic acid (DHA)	Bajpai *et al*., 2009
C. dematium	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. dematium f. *truncatum*	Detoxification of phytolectins	García-Pajón *et al*., 2003
C. destructivum	Detoxification of phytolectins	García-Pajón *et al*., 2003
C. fragariae	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. graminicola	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. gloeosporioides	Detoxification of phytolectins	García-Pajón *et al*., 2003
(as *Glomerella cingulata*)	Transformation of saturated and unsaturated acyclic terpenoids	García-Pajón *et al*., 2003
	Transformation of sesquiterpenes	
	Transformation of widdrol	Kumari *et al*., 2003
		Nunez *et al*., 2006
(as *Glomerella cingulata*)	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. lagenarium	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. lindemuthianum	Reduction of cyclic ketone	García-Pajón *et al*., 2003
C. lini	Steroid hydroxylations	Romano *et al*., 2006
C. trifolii	Detoxification of phytolectins	García-Pajón *et al*., 2003
	Reduction of cyclic ketone	García-Pajón *et al*., 2003

longer becomes a dustbin for gene sequences with the wrong names. At present sequence data for the rDNA-ITS region and a few other genes are available for most of the species with existing type specimens (Hyde *et al*., 2009; Cai *et al*., 2009) so they can provide a name and thus researchers must check their sequences against these type strains to verify the name. In the future it will hopefully be possible to find a better, or even an ideal gene for barcoding species in the genus.

Concluding remarks

Several important papers dealing with the type specimens and cultures of species complexes have been published (Shenoy *et al*., 2007a,b; Cannon *et al*., 2008; Than *et al*., 2008; Crouch *et al*., 2009a-c; Damm *et al*., 2009; Shivas and Tan, 2009). These complexes can now be further studied and unraveled so that we can really begin to understand the genus beyond the species complexes. Whether the complexes can be split into numerous species, a few species with many subspecies, varieties or forms, or just into subspecies, varieties or forms will remain a matter for debate, much of which depend on personal preference rather than scientific evidence. It is hoped that new species introductions will at least adopt the "genealogical concordance method" (Taylor *et al*., 2000) and more (see Cai *et al*., 2009). There is an urgent need for agreement on protocols to handle the naming and describing of types, epitypes, new species, subspecies, varieties and forms, which is the subject of a separate paper in this issue (Cai *et al*., 2009). These proposed recommendations may or may not be followed in the short term, but in the long term *Colletotrichum* specialists should unite and derive a set of technical protocols that must be followed. Until then we predict that over the next 1-2 years there will be many new taxa of *Colletotrichum* named, resulting in perhaps 100-200 accepted species names; there will be numerous name changes to important plant pathogens (e.g. *C. capsici* – see Damm *et al*., 2009), which will no doubt confuse and frustrate plant pathologists; there will be no consensus on which genes to use and different sets of genes will be used, albeit with some overlapping; there will be no standardized descriptions of species, and illustrations will range from a few spores only, to comprehensive full colour plates; pathogenicity testing will be included in a few cases only. We predict that in 2-5 years *Colletotrichum* taxonomy will become more settled; fewer new taxa will be introduced and there will be fewer name changes; plant pathologists will confidently accept the new names; sets of genes for use in species description will be agreed upon and a single magic barcoding gene may be identified;

species descriptions will become standardized (or if morphological characters prove to be totally inadequate they may be done away with altogether); and pathogenicity testing will become essential to establish the biological role of these fungi.

Colletotrichum has particular biosecurity importance and until the genus becomes settled we suggest that plant quarantine authorities delay taking imprudent action. Many *Colletotrichum* species are proving to be widespread on a broad range of hosts and some are proving to be endophytes as well as pathogens. Many of the recently described species (e.g. in Prihastuti *et al*., 2009; Yang *et al*., 2009) are proving to be widespread on a range of unrelated hosts, some as endophytes, epiphytes and pathogens, or as weak or opportunistic pathogens. Thus, besides existing species, such as *C. kahawae*, which is a species of quarantine significance and an important pathogen of coffee in Africa causing a devastating coffee berry disease, we suggest that some *Colletotrichum* species may prove to be cosmopolitan in distribution and we do not need to be overly concerned about them. If countries wish to clarify which species they have within their boundaries, they need to fund the research that will provide the answers. This research will be based on the collection of fresh specimens and DNA sequence data.

Although the many name changes within the genus *Colletotrichum* over the next few years may be confusing, it is necessary to establish a long-term stable taxonomic framework. The examples above illustrate numerous cases where we have no idea whether or not correct names were used. The situation is exacerbated by having approximately 86% of names on GenBank under *C. gloeosporioides* apparently wrongly identified. It is impossible to repeat or extend scientific work and compare findings with existing published work if the names applied to species in publications are erroneous. We have no option but to restudy, revise and clarify the genus, so that the naming of *Colletotrichum* species will, in the future, be reproducible and precise.

Acknowledgements

This research was made possible by grant number 51101010029 and 52101010002 from Mae Fah Luang University.

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