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REVIEW

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Coelomycete systematics with special reference to *Colletotrichum*

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Abstract Morphological and molecular data of coelomycetes are analyzed. Taxonomic tools and species concepts are explored. The bimodal systematics approach is emphasized. A comprehensive case study on *Colletotrichum* is included.

Key words Coeloanamorphoses · Genetic diversity · *Glomerella* · Isozyme · rDNA polymorphisms

Introduction

This article presents a mini-overview of the ongoing saga of coelomycete systematics. It evaluates morphological and molecular data, elaborates upon species concepts in *Colletotrichum*, and advocates a bimodal or dual approach to taxonomy of the coeloanamorphoses.

Morphology and taxonomy

Coelomycetes are deuteromycetes or fungi imperfecti with acervuli, pycnidia, or stromata as their conidiomata (Sutton 1980). These fungi are mitosporic, microscopic, ubiquitous, parasitic, saprobic, or facultative. They generally exist as conidial, spermatial/microconidial states or anamorphs of ascomycetes or basidiomycetes, and might as well be called Fungi Mitospori or Fungi Anamorphoci.

For lack of sexual (perfect/meiospore) stages in their life cycles, coelomycetes are usually consigned to a form-class/division of Deuteromycota (also Dikaryomycota) or Fungi Imperfecti, which is polyphyletic, artificial, and untenable or redundant (Taylor 1995). Form-taxa are classified solely on their conidial ontogeny, morphology, or ecology. Such classificatory systems are exclusive, restrictive, of limited use, and not indicative of clear relationships among taxa. Primarily, they serve nomenclature and identification purposes only.

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Descriptive and diagnostic systematics

Among coelomycetes, *Colletotrichum* is the single genus that has garnered most attention, probably because of the diversity, distribution, and devastation represented particularly by *C. acutatum*, *C. gloeosporioides*, *C. graminicola*, and *C. falcatum* (Thaung 1970). The first three are cumulative species containing disparate groups of strains and biotypes (Kulik et al. 2005).

Colletotrichum is an important phytopathogen typically with setose acervuli, relatively large cylindrical or falcate phialoconidia, and appressoria, attacking a very broad range of host plants. It embraces some 40 species of plant parasites, and provides anamorphs of *Glomerella* with a large reservoir of synonymy too cumbersome for accurate and conclusive systematic study (von Arx and Müller 1954; von Arx 1957).

Traditionally, *Colletotrichum* species are diagnosed and delimited mainly on the morphology of the organism in vivo and/or in vitro and commonly named after the host to denote their source or to suggest specificity. Although not quite effective, traditional methodology has successfully separated morphologically greatly similar and hardly distinguishable cumulative species, especially *C. acutatum* and *C. gloeosporioides* (Smith and Black 1990; Sutton 1992; Jayasinghe et al. 1997; Förster and Adaskaveg 1999), found in close associations on a worldwide scale.

Molecular methodology has therefore come to its aid in the identification process. A number of *C. gloeosporioides* isolates from some fruit and leaf anthracnose diseases have been redetermined as *C. acutatum* by employing molecular

markers and polymerase chain reaction (PCR) diagnostics (Brown and Soepena 1994; Brown et al. 1996; Smith et al. 1996; Martín and García-Figueres 1999; Peres et al. 2002; Saha et al. 2002).

Nevertheless, species names of *Colletotrichum* are no longer of much significance because of their extensive host range, intermediate forms, and morphological and pathological variations (Du et al. 2005) relative to environmental influences (Bailey and Jeger 1992; Freeman et al. 2000).

Molecular techniques and targets in coelomycetes

Molecular assays of coelomycetes started around 1990 with ribosomal DNA (rDNA) furnishing a molecular taxonomic marker (Braithwaite et al. 1990) for rapid and detailed genetic analysis of polymorphisms—variation within a DNA or RNA sequence—in the group species *Colletotrichum gloeosporioides*. A wide array of molecular techniques has since been applied to differentiate isolates of coelomycetes in genera *Colletotrichum*, *Discula* (Trigiano et al. 1995), *Pestalotiopsis* (Jeewon et al. 2002; Sousa et al. 2004; Keith et al. 2006), and *Phomopsis* (Meijer et al. 1994; Rehner and Uecker 1994; Zhang et al. 1997a, 1998, 1999; Uddin et al. 1998; van Niekerk et al. 2005) from various geographic origins and hosts, including humans (Cano et al. 2004).

These procedures together with the targets they probe comprise isozyme analyses of 11, 13, or 19 enzyme systems on the protein level (Bonde et al. 1991; Meijer et al. 1994; Kaufman and Weideman 1996); sequence analyses of the PCR-amplified internal transcribed spacer (ITS) region of rDNA (Mills et al. 1992; Rehner and Uecker 1994; Sherriff et al. 1994, 1995; Sreenivasaprasad et al. 1996; Johnston and Jones 1997; Zhang et al. 1997a; Uddin et al. 1998); amplified fragment length polymorphisms (AFLPs) of genomic DNA (Majer et al. 1996; O'Neill et al. 1997); restriction fragment length polymorphisms (RFLPs) of rDNA, mitochondrial DNA (mtDNA), and genomic DNA (Braithwaite et al. 1990; Vaillancourt and Hanau 1992; Hodson et al. 1993; Alahakoon et al. 1994; Riccioni et al. 2003); RFLPs and DNA–DNA hybridization (Liyanage et al. 1992; Bernstein et al. 1995; Brown et al. 1996); primer PCR-initiated random amplified polymorphic DNAs (RAPDs) or AT-rich DNA analyses of genomic DNA (Welsh and McClelland 1990; Williams et al. 1990; Guthrie et al. 1992; Vaillancourt and Hanau 1992; Freeman et al. 1993; Blakemore et al. 1994; Trigiano et al. 1995; Fernandez and Hanlin 1996; Freeman and Katan 1997; Mackie and Irwin 1998; Chakraborty et al. 1999); species-specific primer analysis of rDNA-ITS1 region (Brown et al. 1996; Adaskaveg and Hartin 1997; Freeman et al. 2000); and high mobility group (HMG)-encoding sequence of the *MAT1–2* mating type sequence (Du et al. 2005).

Furthermore, vegetative compatibility group (VCG) analysis offers a system ancillary or collateral to RAPD for exploration of genetic distinctness or diversity and relationships between several morphological groups recognized within a pathogen population that appears to be genotypically homogeneous by molecular assays (Lardner et al. 1999; Freeman et al. 2000; Katan 2000; Nitzan et al. 2002).

Molecular approach to *Colletotrichum* species

Colletotrichum species were grouped or classified utilizing RFLPs generated by the endonucleases *Mvn*I, *Pvu*II, and *Scr*FI (Martínez-Culebras et al. 2000), multiple loci–nucleotide sequence data from mitochondrial small subunit rDNA (mtSSU) and a fragment of β-tubulin gene sequencing combined with ITS sequencing (Vinnere 2004), β-tubulin 2 nucleotide sequences (Talhinhas et al. 2005), rDNA-ITS sequencing combined with partial sequences of the β-tubulin gene (Lubbe et al. 2004), and rDNA-ITS1 sequencing (Moriwaki et al. 2002; Photita et al. 2005). These assays produce results conforming to traditional morphological, cultural, physiological, and pathogenic characters of the taxa.

Colletotrichum species complexes such as *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, and *C. graminicola* have been delineated using species-specific PCR primer analysis (Freeman et al. 2000; Afanador-Kafuri et al. 2003; Kulik et al. 2005), PCR-RFLP and/or sequence analysis of the rDNA (ITS1-5·8S-ITS2) (Martín and García-Figueres 1999; Martínez-Culebras et al. 2000; Abang et al. 2002; Guerber et al. 2003), RAPD-rDNA (Kuramae-Izioka et al. 1997; Saha et al. 2002), HMG-encoding sequence of the *MAT1-2* mating type sequence and rDNA sequences (Du et al. 2005), arbitrarily primed-PCR-β-tubulin 2 nucleotide sequence analysis of rDNA-ITS and AFLP analysis (Sreenivasaprasad et al. 1994; Talhinhas et al. 2002, 2005), and isozyme analysis (Buddie et al. 1999).

Regions of rDNA and results

Regions of rDNA selected for molecular analysis potentially differ in their productivity, diversity, and utility of data relevant to the investigation. The rDNA-ITS1 region shows a greater degree of intra- and interspecific divergence than the rDNA-ITS2 region, and the rDNA-ITS1 data can quickly and confidently not only identify but also infer phylogenetic relationships between *Colletotrichum* species (Sreenivasaprasad et al. 1994, 1996). On the other hand, the entire rDNA (ITS1 + ITS2) region is more informative of diversity than that of the rDNA-ITS1 region (Freeman et al. 2001).

rDNA-ITS1 sequence data confirmed the morphometric distinction between *C. graminicola* and *C. sublineolum* based on the structure of in vitro appressoria and host specificity (Sherriff et al. 1995), and unambiguous separation as well among *C. coccodes*, *C. crassipes*, *C. dematium*, *C. gloeosporioides*, and *C. graminicola* based on sclerotia, appressoria, and conidia in culture (Cano et al. 2004). The

molecular and morphological data are, for the most part, concordant in such instances. Similarly, nucleotide sequence analyses of the rDNA (ITS1-5.8S-ITS2) region in *Pestalotiopsis* demonstrated phylogenetic significance of morphological characters as well as a congruence between molecular and morphological data on pigmentation of median cells and apical appendage tip shape (Jeewon et al. 2002, 2003).

However, morphological data in some *Colletotrichum* species or species clusterings were occasionally not in accord with the molecular data obtained from their rDNA-ITS sequences (Sherriff et al. 1994; Sreenivasaprasad et al. 1996; Munaut et al. 2002) or the rDNA large subunit (LSU) D2 domain sequence (Johnston and Jones 1997). rDNA-ITS sequence homology and "species-specific" primers afford cases where molecular criteria do not quite coincide with morphological characteristics of *Colletotrichum* isolates from lupin. This discordance could be attributed to their limited capacity to detect genetic variations, in contrast to RAPD analysis. Confusion can ensue from systematic conclusions based on such anomalous data. Consequently, doubts have been cast about rDNA-ITS assays being the only tools for *Colletotrichum* species separation (Yang and Sweetingham 1998). Alternative techniques are yet available to dispel such doubts and demonstrate a conformance between the two.

Alternative or comparative assays

ITS sequence performance tests for morphological concurrence encompass RFLP-rDNA (ITS1-5·8S-ITS2) analysis (Martín and García-Figueres 1999), arbitrarily primed (ap) PCR and AT-rich DNA analyses (Freeman et al. 2001), and the *MAT1-2* mating type sequence (Du et al. 2005).

Arbitrarily primed PCR data, rDNA-ITS gene and β-tubulin 2 nucleotide sequences, fit the global populations of *C. acutatum* into at least eight distinct molecular groups, A1–A8, which correlate, to some varying degree, with the morphological parameters, host association, and geographic distribution (Sreenivasaprasad and Talhinhas 2005; Talhinhas et al. 2005). Primed PCR and AT-rich DNA analysis groups *Colletotrichum* isolates into ten separate species on the similarity of interspecific data consisting of *C. acutatum*, *C. coccodes*, *C. fragariae*, *C. gloeosporioides*, *C. lindemuthianum*, *C. magna*, *C. musae*, *C. orbiculare*, and *C. graminicola* from maize and *C. sublineolum* from sorghum. In general, groupings by molecular data correspond to those obtained by traditional taxonomic identifications, although there are some exceptions (Freeman et al. 1993).

RAPD-PCR banding patterns and rDNA-ITS sequence analysis recently diagnose, differentiate, and characterize two varieties—*Colletotrichum lupini* var. *lupini* and *C. lupini* var. *setosum*—in modern descriptive terms, of RAPD group 1 for *C. lupini* var. *lupini* and RAPD group 2 for *C. lupini* var. *setosum* (Nirenberg et al. 2002). PCR-rDNA-ITS and β-tubulin sequence data were similarly used to profile and prop up the name of *C. lupini* (Lotter and Berger 2005).

Conflict resolutions

rDNA-ITS sequence analysis sometimes generates molecular data not in agreement with morphological data or not confirmable through the rDNA–(LSU = 28S)–D1-D2 domain test.

In case of a conflict between molecular and morphological systematic data, the latter prevails for a taxonomic species is a rank-based, legitimate entity defined in standard morphometric parameters in Latin as governed by the International Code of Botanical Nomenclature. Incidentally, this Linnaean species concept stands in stark contrast to that of a phylogenetic code (PhyloCode) where a species is the smallest monophyletic clade following a different naming system. Both codes can be quoted concurrently (www.ohiou.edu/phylocode/).

In the event of a clash or asymmetry in results between molecular methods as, for example, ITS1 sequence homology and RFLP, RAPD, or AFLP, the latter triumphs as they can detect greater genetic variation, characterize pathogen variability or several pathosystems, find fingerprints or markers, and also consistently yield results compatible with classic taxonomic and pathogenic criteria (Braithwaite et al. 1990; Freeman et al. 1993; Hayden et al. 1994; Pastor-Corrales et al. 1995; O'Neill et al. 1997).

Colletotrichum systematics

In view of the time, labor, cost, contamination, and potential risk of error (Camacho et al. 1997; Zhang et al. 1997b) inherent in the process of securing genomic data for use in molecular taxonomy, morphological criteria of a taxon plus its overall biology and ecology should be taken into account in advancing the current *Colletotrichum* species concept (Vinnere 2004; Wharton and Diéguez-Uribeondo 2004). Conversely, molecular augmentation of, or integration with, traditional morphological classification as well as pathological information could evolve into an effective system in *Colletotrichum*, appropriating all available resources (Cannon et al. 2000; Abang et al. 2003). Accordingly, both traditional and molecular methods identified and characterized *Pestalotiopsis* species—*P. clavispora*, *P. microspora*, *P.* species GJ-1, and *P. disseminata* causing scab on guava in Hawaii (Keith et al. 2006).

Molecular, morphological/cultural, and pathogenic data together suggest a trinomial group-species concept (Johnston and Jones 1997) or polynomial system (Cannon et al. 2000) within *Colletotrichum* with a taxonomic infraspecific differentiation such as *C. gloeosporioides* f. *stylosanthis* f. sp. *stylosanthis* and f. sp. *guianensis* (Munaut et al. 2002). The species name defined on rDNA sequence similarity and supporting morphological/cultural features indicates a large group within *Colletotrichum* to which the isolate belongs; the subspecific name imparts additional information on host specialization, morphological or ecological variation, etc. (Johnston and Jones 1997). The competitions are a dual

nomenclatural system, one for applied mycology and the other for pure mycology (Cannon et al. 2000), and a phylogenetic species concept to accommodate pathogenic fungi whether asexual or sexual (Taylor et al. 1999).

Observations

Molecular means deployed to distinguish the *Colletotrichum* species complex are chiefly PCR assays with primers detecting DNA fingerprints as RFLP-mtDNA, RFLP-rDNA (ITS1-5·8S-ITS2) ± endonucleases *Mvn*I, *Pvu*II, and *Scr*FI, RFLP-rDNA (18S + 28S), RAPD-rDNA, β-tubulin 2 nucleotide sequence-rDNA-ITS1 region, and sequence analyses of rDNA (ITS1 ± ITS2) and (D2) regions, (HMG)-encoding sequence of the *MAT1-2* mating type sequence. PCR processes are more reliable, widely used as diagnostic tools, and capable of detecting pathogenic fungi directly in plant tissue. Illustrations include RFLP-rDNA-ITS to distinguish *Diaporthe phaseolorum* and *Phomopsis longicolla* from other soybean pathogens, RFLP-rDNA-ITS with species-specific PCR primer to detect the two fungi in soybean plants and seeds, and RAPD-rDNA to differentiate *D. phaseolorum* var. *meridionalis* isolates from *Macrophomina phaseolina* and *Phoma* species.

However, rDNA-ITS1 sequence analysis supplies an additional, potentially useful tool to determine and differentiate *Colletotrichum* species without using morphology as it proves to be efficient and sensitive and delivers results of correlation between morphological and molecular-based clusterings.

Phylogenetic relationships have been inferred for *Colletotrichum*, *Pestalotiopsis*, and *Phomopsis* species from (1) nucleotide sequences in the rDNA (ITS1-5.8S-ITS2) region, (2) analysis of rDNA (ITS2 + 28S D2) region, (3) sequence variation in rDNA (ITS1 + ITS2) regions, (4) RFLP maps from differential restriction of the rDNA (ITS1 + ITS2) region, (5) PCR-rDNA (ITS1 + ITS2) sequence, and (6) *HMG*-encoding sequence of the *MAT1-2* mating type sequence. rDNA-ITS loci are taxonomically informative of relatedness among genera or between fungal strains, although not sufficiently diverse to allow full resolution of closely related lineages.

This overview highlights the importance and excellence of molecular techniques as applied to coelomycetes phylogenetics and systematics. The methodology has contributed to elucidation of the diversity, phylogeny, and taxonomy of *Colletotrichum* by assessing molecular variations and deciphering the genetic relationships among taxa. The molecular data can be exploited to complement identification based only on morphological criteria, which in many cases, is still the most common technique. An augmented or integrated taxonomic approach is in order to better understand the pathogen identity, host gamut, population dynamics, and epidemics for improved disease control (Dron and Bailey 1999).

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