2016

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Dugan, Frank M. and Everhart, Sydney, "Cryptic Species: A Leitmotif of Contemporary Mycology Has Challenges and Benefits for Plant Pathologists" (2016). *Papers in Plant Pathology*. 425.
http://digitalcommons.unl.edu/plantpathpapers/425

Plant Health Review

Cryptic Species: A Leitmotif of Contemporary Mycology Has Challenges and Benefits for Plant Pathologists

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Accepted for publication 16 November 2016.

ABSTRACT

Multiple traditional species names for plant pathogenic fungi have been supplemented with new names that delimit formerly cryptic species. In separate instances, isolates within a species are clearly differentiated by both phylogeny and distinctive pathogenic traits and are assigned sub-specific designations. These new species names and the sub-specific designations are both cases of cryptic species that are, in some instances, relevant and/or critical for plant disease management. Here we provide examples of such instances in which newly described taxa differ from the original ("parent") in phenotypic traits of importance to plant disease management: host range, fungicide sensitivity, environmental niche, metabolite production, regulatory status, or other attributes.

INTRODUCTION

Plant health professionals apply various approaches to disease management. Especially notable are epidemiology and forecasting; chemical, biological, and cultural disease suppression; breeding and disease resistance; diagnostics; and regulatory activities. Persons in the profession typically specialize in one of these fields while maintaining a basic, working understanding in all these sub-disciplines. Consequently, it is frustrating to many plant pathologists that names attached to plant pathogens, especially to fungal agents of plant disease, are subject to seemingly endless nomenclatural and taxonomic revision. To determine the degree to which such revisions assist or impede progress on the approaches just specified, it is important to assess (i) the degree to which these revisions assist more accurate determination of identity of disease agents and (ii) the degree to which agents formerly treated as identical (i.e., lumped into a single, "parent" species, but now regarded as distinct) differ in behavior, including response to management strategies.

An increasing number of new species names are published on the basis of gene typologies. These new species are often referred to as cryptic species because they were previously not regarded as phylogenetically distinct, or if so, insufficiently distinct for separate species status. Sometimes, problems are detected with regard to conformity to other species concepts, e.g., Harrington et al. (2014) on conflicts between phylogenetic and biological species in *Ceratocystis*. From such instances, it might be concluded that not all new species are justified from the standpoint of practical plant pathology.

In this manuscript, we review five case studies in which newly described fungi (or in one case, a pseudofungus) differ markedly from their "parent" species in ways that imply changes to plant disease management. These primary differences, and corresponding parent species, are: (i) environmental preference and mycotoxin production (parent, *Aspergillus niger*); (ii) host range and fungicide susceptibility (parent, *Botrytis cinerea*); (iii) host range and mycotoxin production (parent, *Fusarium subglutinans*); (iv) host range (parent, *Penicillium corymbiferum*); and (v) host specificity (parent, the oomycete *Plasmopara viticola*).

CASE SUMMARIES

***Aspergillus niger*.** Different species of black aspergilli (*Aspergillus* section Nigri, ~30 spp.; Varga et al. 2011), important in decay of various fruits, bulbs, and other plant products, may differ in mycotoxin production, with repercussions in human/animal health and in production of fermented foods/beverages. For example, various strains of *A. carbonarius*, *A. niger*, and *A. welwitschiae* (= *A. awamori* on the basis of type cultures, specimens used when describing a species; Hong et al. 2013) produce a mycotoxin, ochratoxin A, whereas strains of *A. niger* and *A. welwitschiae* may also produce the mycotoxin fumonisin B_2 (Hong et al. 2013; Palumbo and O'Keeffe 2014).

The fungus described as *A. awamori* by Perrone et al. (2011) was renamed *A. welwitschiae* (Hong et al. 2013) because it is identical to type material isolated from *Welwitschia* (an African gymnosperm). Strains of *A. welwitschiae* are toxin-producers, and are not closely related to the fungi used in awamori koji fermentation, a production process used for several traditional Asian foods such as soy sauce (Palumbo and O'Keeffe 2014). The phylogenetic species *A. awamori* sensu Perrone et al. does *not* correspond with isolates of that name used in production of koji. A representative isolate used in awamori koji production was selected as the type culture of *Aspergillus acidus* (= *A. lucheunsis*), which does not produce mycotoxins and is considered safe for food and beverage fermentation (Hong et al. 2013).

In addition to differing in metabolites, segregates from *A. niger* may differ in environmental niche adaptation. Although very similar or indistinguishable morphologically and with relatively broad host ranges, some species of the Nigri complex appear preferentially adapted to certain ecological niches; e.g., *A. tubingensis* predominated over other members of the complex in Spanish vineyards (García-Cela et al. 2014). Differences between

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doi:10.1094/PHP-RV-16-0046

A. niger and *A. awamori* (sensu Perrone et al. 2011) in geographic locale and host preference are described by Varga et al. (2011). Thus species names, including names of new, formerly cryptic, species of black aspergilli convey information on environmental preference and toxin production.

***Botrytis cinerea*.** *Botrytis cinerea*, causal agent of gray mold, is a destructive pathogen on over 200 hosts of agronomic importance, and is especially important on grapes (Williamson et al. 2007). New (formerly cryptic) species are increasingly described, sometimes with evident management implications. For example, populations found to have reduced sensitivity to fenhexamid (FRAC code 17), a fungicide used for disease control, were determined to be different species, *B. californica* and *B. pseudocinerea* (Saito et al. 2014a, 2014b, 2016). Similarly, *B. sinoviticola*, a formerly cryptic species clustering phylogenetically with *B. cinerea* and *B. pseudocinerea*, is less sensitive to fenhexamid than is *B. cinerea* (Zhao et al. 2014).

Two other examples include *Botrytis caroliniana*, described from blackberry (*Rubus fruticosus*) in South Carolina, which differs from *B. cinerea* (common on blackberry) in cultural characters on media and from its closest phylogenetic relatives, *B. galanthina* and *B. fabiopsis*, in host range (Li et al. 2012). *Botrytis pseudocinerea* appears, like *B. cinerea*, to have a wide host range but is subject to replacement by fungicide-resistant strains of *B. cinerea*, and appears to produce fungicide-resistant strains less frequently than *B. cinerea* (Plesken et al. 2015). Both host range and fungicide susceptibility/resistance play strong roles in disease management.

***Fusarium subglutinans*.** Phylogenetic research on the *F. subglutinans* complex has shown it to include more than 20 species, and new species continue to be described, e.g., *Fusarium ficicrescens*, described by its authors as a cryptic species within *F. subglutinans* (*Gibberella fujikuroi*), recently described from spoiled figs, *Ficus carica* (Al-Hatmi et al. 2016). The complex includes important plant pathogens such as *F. sacchari* (especially notable on sugarcane, *Saccharum officinarum*), *F. circinatum* (causing pitch canker on *Pinus* spp.), and *F. mangiferei* (causing a deformation in mango, *Mangifera indica*). The latter two are of quarantine importance in Australia (Hyde et al. 2010).

An analogous but separate instance of historical importance is the name *F. moniliforme* (now regarded as part of *Gibberella fujikuroi*, mating population A), once applied to at least three species, *F. verticillioides*, *F. thapsinum*, and *F. andiyazi*. Also included are a number of currently undescribed taxa, all morphologically identical but differing among themselves biologically, ecologically, and phylogenetically. *Fusarium verticillioides* causes stalk rot of maize (*Zea mays*) and produces fumonisin, whereas *F. thapsinum* causes stalk rot of sorghum (*Sorghum bicolor*) and does not produce fumonisin. *Fusarium andiyazi* can also cause some disease in sorghum (Hyde et al. 2010). Host preference and mycotoxin production are strong considerations in management of *Fusarium*-induced diseases of cereals and other crops.

Penicillium corymbiferum. For most of the twentieth century, *Penicillium* isolates causing blue mold of edible and ornamental bulbs were lumped into a single species, *P. corymbiferum* (= *P. hirsutum*). Segregates of this species, several first as varieties and later elevated to species, comprised series *Corymbifera* in subgenus *Penicillium* (Frisvad and Samson 2004). *Penicillium corymbiferum* was long held to be the pathogen responsible for blue mold of garlic (*Allium sativum*), and several ornamental bulbs (Dugan et al. 2014). However, more recent studies identified differences in metabolite production (Overy et al. 2005a), host range (Dugan et al. 2014), and environmental preference (Overy 2005b) among the segregate species.

"With the exception of *P. allii*, all six of the remaining ser. *Corymbifera* taxa have a potential to proliferate at low temperatures and would therefore cause disease under storage conditions" (Overy et al. 2005b). *Penicillium allii* is the dominant *Pencillium* species attacking garlic in the field and at higher temperatures (Dugan et al. 2011), although garlic is susceptible to attack by some (not all) members of ser. *Corymbifera* (Dugan et al. 2014). *Penicillium allii*, *P. hirsutum*, *P. tulipae*, and *P. venetum* (all in series *Corymbifera*) differed in pathogenicity and aggressiveness on crocus (*Crocus sativus*), daffodil (*Narcissus*), gladiolus (*Gladiolus*), grass lily (*Ornithogalum umbellatum*, Fig. 1), tulip (*Tulipa*), and two varieties of table onion (*Allium cepa*) (Dugan et al. 2014). Thus *Penicillium* spp. inducing blue mold of bulbs can differ markedly in host preference, a consideration in management by host rotation.

***Plasmopara viticola*.** *Plasmopara viticola*, the pseudofungal agent of grape downy mildew, is one of the most important diseases of grape worldwide (Gessler et al. 2011). Recent advances have demonstrated that this taxon is composed of multiple cryptic species, each indicated in Rouxel et al. (2013) as a *forma specialis*: *Plasmopara viticola* f. sp. *quinquefolia*, f. sp. *vinifera*, f. sp. *aestivalis*, and f. sp. *riparia*. Each has distinct to partially overlapping host ranges in *Vitis* and *Parthenocissus*, both members of the family Vitaceae (Rouxel et al. 2013). Although naming the taxa as *formae speciales*, the authors explicitly refer to them as cryptic species and demonstrate phylogenetic separation on the basis of multiple gene topologies. “Some previous research on *P. viticola* will need to be revisited, because it was unclear which species were under study” (Rouxel et al. 2013).

Isolates on *Parthenocissus tricuspidata* (grape ivy) in North America appear to belong to *Plasmopara muralis*, a species described from Germany. The *formae speciales* of Rouxel et al. (2013) are “clades” in Rouxel et al. (2014). Novel taxon *P. viticola* clade *vulpina* is restricted to *Vitis vulpina*, fox grape (Rouxel et al. 2014). Rouxel et al. (2013) discuss implications, including breeding for resistance, the importance of understanding spatiotemporal distributions of these cryptic species, and accurate identification.

FIGURE 1

Penicillium venetum ATCC 36410 (top right) and *P. hirsutum* NRRL 999 (bottom right) inoculated to bulbs of *Ornithogalum umbellatum* (grass lily); controls are on the left. *Penicillum hirsutum* (sensu stricto) and *P. venetum* (formerly *P. hirsutum* var. *venetum*) are segregates of the “parent” species, *P. corymbiferum*, and differ both phylogenetically and in host range. (Photo credit: Shari Lupien).

DISCUSSION AND CONCLUSIONS

Cases summarized above are extracted from a much wider literature. Other recent examples of cryptic species or suspected cryptic plant pathogenic species include taxa potentially segregated from *Monilinia vaccinii-corymbosi*, a pathogen of blueberry (*Vaccinium* spp.) (Burchhardt and Cubeta 2015) and species within *Claviceps purpurea*, agent of ergot (Pažoutová et al. 2015). Examples from natural environments include cryptic species within *Lophodermium*, needle-inhabiting fungi in *Pinus* spp., i.e., *Lophodermium australe* (Oono et al. 2014) and *L. pinastri* (Reignoux et al. 2014); and a potential cryptic species within *Armillaria gallica*, one of several agents of root disease of forest trees, especially conifers (Ross-Davis et al. 2012). Plant pathologists with mycology as a sub-discipline are not uniquely affected. Analogous situations occur in nematology (Palomares-Ruis et al. 2014), entomology (Green et al. 2013), and bacteriology (Goss et al. 2005).

Methods used in delimiting these formerly cryptic species were not addressed in detail here. Multi-gene topologies are widely accepted, but not invariably successful at identifying taxa with attributes of primary interest to plant pathologists. Barcoding (identification on the basis of a single gene sequence) should be avoided if a given locus could be affected by horizontal gene transfer, as is the case with *Aspergillus* and *Penicillium* (Peterson 2012). It seems unlikely that a single method will be universally applied for species determination, including discovery of formerly cryptic species, across all groups of plant pathogenic fungi and pseudofungi. Although some have suggested that whole-genome sequence analysis will eliminate this issue, mounting evidence suggests not.

Questions remain: What species concepts for disease agents are most useful for plant disease management? Will the species concepts most useful for disease management differ between taxonomic groups? Are sub-specific designations more useful? These are not merely academic questions. Naming of plant pathogenic fungi is of prime importance in matters of trade and quarantine (Crous et al. 2015), but controversy attends the application of species concepts (Taylor et al. 2000; Taylor et al. 2006), and modern species concepts have even been called a failure (Hey 2006).

Cases summarized here are representative, though hardly exhaustive. The uniting feature in each case is that the newly described species or subspecies differ significantly from the parent species and is different enough that management recommendations for the parent species must be modified when addressing the new species. For practicing plant pathologists, this is the bottom line for determining whether a formerly cryptic taxon is "real" or of exclusively academic interest. These formerly cryptic but very real taxa must be integrated into epidemiology and forecasting, chemical, biological and cultural disease suppression, breeding and resistance, diagnostics, and regulatory approaches.

ACKNOWLEDGMENTS

The authors thank Robert Harveson and Rachel Bomberger for constructive comments on the manuscript, and Shari Lupien for technical assistance.

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