Cryptic fungal species unmasked

Roger G Shivas[1] *& Lei Cai*[2]

1 Plant Biosecurity Science, Department of Employment, Economic Development and Innovation (DEEDI)
GPO Box 267, Brisbane QLD 4001
Tel (07) 3255 4378
Fax (07) 3844 9716
Email roger.shivas@deedi.qld.gov.au

2 State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, HaiDian District, Beijing 100190, People's Republic of China
Tel/Fax + 86 10 6256 0523
Email mrcailei@gmail.com

The word cryptic is derived from the Greek adjective *kruptós* which means hidden. Morphologically indistinguishable species that have been revealed by molecular phylogenetic methods, and ultimately only recognised by their DNA sequences, are referred to as cryptic species. The importance of cryptic species for plant pathologists is that they may have significant differences in the severity of diseases they cause, host range and geographic distribution. It is these differences that are of concern to many biosecurity agencies, particularly in Australia.

There has been a rapid increase in the number of cryptic species of plant pathogenic fungi discovered in recent years with the widespread use of DNA sequence-based techniques[1,2] and the application of the genealogical concordance phylogenetic species recognition criterion[3]. In the six years since Crous and Groenewald declared *Show me a plant pathogen, and I will show you a species complex*[4], there has been much supportive evidence provided that many current names of common and widespread plant pathogens mask complexes of cryptic species.

Colletotrichum gloeosporioides is perhaps one of the best examples of an apparently common and widespread plant pathogen that has been shown to hide several cryptic species[5]. Since it was first described as *Vermicularia gloeosporioides* more than 120 years ago[6], *C. gloeosporioides* has been linked as a pathogen to more than 470 different genera of host plants[7]. It was considered the cause of anthracnose of leaves and fruits of many tropical plants. Molecular phylogenetic analysis has shown that *C. gloeosporioides* represents a complex of species that are currently the subject of intensive research[8]. Recently many species of *Colletotrichum* have been shown to represent complexes of cryptic species, including *C. acutatum*[9] and *C. dematium*[10].

Many genera of fungi contain well-known and important species of plant pathogenic fungi that actually represent complexes or aggregates of cryptic species[11]. Examples from both the Ascomycota and Basidiomycota include *Calonectria*[12], *Diaporthe* (incl. *Phomopsis*) [13], *Dothiorella*[14], *Fusarium*[15], *Phyllosticta* (incl. *Guignardia*)[16], *Melampsora*[17] and *Microbotryum*[18].

In many countries the accurate identification of plant pathogenic fungi is of particular importance to their national biosecurity agencies, which have a mandate to prevent or delay the introduction of exotic pests and pathogens. The value of recognising cryptic species is demonstrated by two examples from some recent work in Australia.

Three cryptic species of *Diaporthe* associated with stem canker of sunflower in Queensland and New South Wales have been discovered and named[19]. These three cryptic species differ from *Diaporthe helianthi* that is exotic to Australia and causes a serious disease on sunflowers in Europe. This finding highlighted the need for continued vigilance to prevent the introduction of *D. helianthi* into Australia.

Two cryptic species of *Peronosclerospora*, which cause downy mildew on native *Sorghum* spp. in northern Australia, have been described[20]. One of these cryptic species, *P. australiensis*, is also found on cultivated maize. Prior to the discovery of these cryptic species, many Australian specimens of *Peronosclerospora* on maize had been identified as *P. maydis*, which is one of the most destructive diseases of maize in Indonesia[21]. There is a possibility that *P. maydis* does not occur in northern Australia, in which case it would pose a biosecurity threat to the maize and sorghum industries in Australia.

The recognition of cryptic species and consequent reclassification and proliferation of newly named species has caused difficulty for applied biologists and plant pathologists who work with these organisms. These difficulties are likely to be compounded in the next few years by the formal abandonment from 1 January 2013 of the system of dual nomenclature for fungi with sexual and asexual stages[22]. This very necessary change to the rules of nomenclature was triggered by the ability of molecular techniques to confidently place fungi with no known sexual stage into genera with known sexual stages.

The desire of mycologists and plant pathologists alike is a stable taxonomy for the fungi with which they work. Cryptic species define closely related populations that have separated recently and whose genetic differences may have significant consequences. Only accurate and unambiguous pathogen names will lead to reliable biological information on which sound disease management and biosecurity decisions can be based. DNA barcoding methods[23] offer a screening procedure that may motivate an evaluation of the incidence of cryptic species amongst plant pathogenic fungi.

The unmasking of cryptic species means that some previous plant pathology research will need to be revisited, because it was not clear which species were studied. For example, many plant pathogen records for countries will become obsolete and will need to be reassessed. New opportunities now exist for plant pathologists to determine the agricultural and environmental importance of these newly revealed cryptic species by assessing their host range, pathogenicity and distribution. Unmasking and understanding cryptic species is one of the major challenges for mycologists and plant pathologists in the next decade.

References

1. Cai, L. *et al.* (2011) The evolution of species concepts and species recognition criteria in plant pathogenic fungi. *Fungal Diversity* 50, 121–133.
2. Groenewald, J.Z. *et al.* (2011) Impact of DNA data on fungal and yeast taxonomy. *Microbiol. Australia* 100–104.
3. Taylor, J.W. *et al.* (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genet. Biol.* 31, 21–32.
4. Crous, P.W. and Groenewald, J.Z. (2005) Hosts, species and genotypes: opinions versus data. *Australas. Plant Pathol.* 34, 463–470.
5. Cannon P.F. *et al.* (2008) The typification of *Colletotrichum gloeosporioides*. *Mycotaxon* 104, 189–204.
6. Penzig, O. (1882) Funghi Agrumicoli: contribuzione allo studio dei funghi parasiti degli agrumi, 1–124.
7. Sutton B.C. (1980) The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Commonwealth Mycological Institute, Kew, UK. 696 pp.
8. Hyde, K.D. *et al.* (2009) *Colletotrichum* – names in current use. *Fungal Diversity* 39, 147–183.
9. Shivas R.G. and Tan Y.P. (2009) A taxonomic re-assessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. *Fungal Diversity* 39, 111–122.
10. Damm, U. *et al.* (2009) *Colletotrichum* species with curved conidia from herbaceous hosts. *Fungal Diversity* 39, 45–87.
11. Hyde, K.D. *et al.* (2010) A case for re-inventory of Australia's plant pathogens. *Persoonia* 25, 50–60.
12. Lombard, L. *et al.* (2010) Phylogeny and systematics of the genus *Calonectria*. *Studies. Mycol.* 66, 31–69.
13. Diogo, E.L.F. *et al.* (2010) Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. *Fungal Diversity* 44, 107–115.
14. Phillips, A.J.L. *et al.* (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. *Persoonia* 21, 29–55.
15. Nalim, F.A. *et al.* (2011) New species from the *Fusarium solani* species complex derived from perithecia and soil in the Old World tropics. *Mycologia* 103, 1302–1330.
16. Glienke, C. *et al.* (2011) Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. *Persoonia* 26, 47–56.
17. Bennett, C. *et al.* (2011) Molecular and pathogenic variation within *Melampsora* on *Salix* in western North America reveals numerous cryptic species. *Mycologia* 103, 1004–1018.
18. Le Gac, M. *et al.* (2007) Phylogenetic evidence of host-specific cryptic species in the anther smut fungus. *Evolution* 61, 15–26.
19. Thompson, S.M. *et al.* (2011) Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. *Persoonia* 27, 80–89.
20. Shivas, R.G. *et al.* (2012) *Peronosclerospora australiensis sp. nov. and Peronosclerospora sargae* sp. nov., two newly recognised downy mildews in northern Australia, and their biosecurity implications. *Australasian Plant Pathology*. 41: 125-130. 10.1007/s13313-011-0097-z.
21. Thurston, H.D. (1998) Tropical plant diseases, 2nd edn. APS, St. Paul.
22. Hawksworth, D.L. (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. *IMA Fungus* 2, 155–162.
23. Seifert, K. (2009) Progress towards DNA barcoding of fungi. *Mol. Ecol. Resour.* 9, *Issue Suppl* 83–89.

Biographies

Prof Roger G Shivas is leader of the Plant Biosecurity Science group at DEEDI, Ecosciences Precinct, Dutton Park, Queensland, Australia. His research interests are focused on the systematics of plant pathogenic fungi, especially those of biosecurity significance to Australia. Dr Shivas is a Visiting Professor at the Institute of Microbiology, Chinese Academy of Sciences and on the editorial board of several international journals of plant pathology and mycology.

Prof Lei Cai is a full Professor of mycology at the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, People's Republic of China. His research interests are focused on the biodiversity, systematics and phylogeny of microfungi. Dr Cai was awarded the prestigious Keisuke Tubaki Medal as the first International Mycological Association Young Mycologist Award for Asia for 2010. He is the Executive Associate Editor of *Mycology* – an international journal on fungal biology.