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Phylogenetic species recognition and hybridisation in *Lasiodiplodia*: A case study on species from baobabs

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ABSTRACT

Lasiodiplodia species (*Botryosphaeriaceae*, *Ascomycota*) infect a wide range of typically woody plants on which they are associated with many different disease symptoms. In this study, we determined the identity of *Lasiodiplodia* isolates obtained from baobab (*Adansonia* species) trees in Africa and reviewed the molecular markers used to describe *Lasiodiplodia* species. Publicly available and newly produced sequence data for some of the type strains of *Lasiodiplodia* species showed incongruence amongst phylogenies of five nuclear loci. We conclude that several of the previously described *Lasiodiplodia* species are hybrids of other species. Isolates from baobab trees in Africa included nine species of *Lasiodiplodia* and two hybrid species. Inoculation trials with the most common *Lasiodiplodia* species collected from these trees produced significant lesions on young baobab trees. There was also variation in aggressiveness amongst isolates from the same species. The apparently widespread tendency of *Lasiodiplodia* species to hybridise demands that phylogenies from multiple loci (more than two and preferably four or more) are compared for congruence prior to new species being described. This will avoid hybrids being incorrectly described as new taxa, as has clearly occurred in the past.

Introduction

Species represent the basic units of taxonomy. However, decisions on how to define species boundaries, especially in fungi, are often problematic. Three species concepts are most commonly applied in fungal taxonomy, namely the Morphological (MSR), Biological (BSR) and Phylogenetic Species Recognition (PSR) concepts (Taylor *et al.* 2000) and all three present some challenges. Historically, fungal taxonomy has relied on the MSR concept, where species were described only when they could be distinguished based on distinct morphological characteristics (Taylor *et al.* 2000). The advent of DNA sequencing

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and an ability to apply phylogenetic inference has shown clearly that MSR has substantially underestimated the global fungal diversity (Crous *et al.* 2006; Schoch *et al.* 2014).

The BSR concept postulates that individuals of different species should be reproductively isolated (Taylor *et al.* 2000). However, there are growing numbers of examples where different species of fungi are able to cross and effectively reproduce to form hybrids. For example, a viable interspecies hybrid of *Fusarium circinatum* and *Fusarium subglutinans* has been produced under laboratory conditions (De Vos *et al.* 2011). Other examples include the hybrid poplar rust *Melampsora ×columbiana*, which is a natural hybrid of *Melampsora medusae* and *Melampsora occidentalis* (Newcombe *et al.* 2000), and the hybrids between the white pine blister rust *Cronartium ribicola* and *Cronartium comandrae* (Joly *et al.* 2006). An additional problem with the BSR concept is the fact that many fungi are known only in their asexual states and it is not possible to determine whether they are able to reproduce sexually.

The PSR concept, and more specifically the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept, is increasingly widely used to delineate species of fungi. This approach relies on determining the concordance between multiple gene genealogies and delimiting species where the branches of multiple trees display congruence (Taylor *et al.* 2000). The GCPSR ensures that species are not described based on small differences arising from within taxon variation.

The PSR has been widely applied during the last decade to describe cryptic species that could not be identified using the MSR. One example where a number of cryptic species have been described is in *Lasiodiplodia*, a common genus in the *Botryosphaeriaceae* (Phillips *et al.* 2013). The type species of this genus, *Lasiodiplodia theobromae,* has been reported from more than 500 plant species (Punithalingam 1976). This was, however, before the advent of DNA sequence-based identification (Pavlic *et al.* 2004; Slippers *et al.* 2004; Alves *et al.* 2008; Pavlic *et al.* 2009; Phillips *et al.* 2013). For many years *L. theobromae* was the only species in *Lasiodiplodia*, but 28 additional species have been described since 2004, based on both DNA sequence data and morphological characteristics (Pavlic *et al.* 2004; Burgess *et al.* 2006; Damm *et al.* 2007; Alves *et al.* 2008; Pavlic *et al.* 2008; Abdollahzadeh *et al.* 2010; Begoude *et al.* 2010; Ismail *et al.* 2012; Liu *et al.* 2012; Urbez-Torres *et al.* 2012; Machado *et al.* 2014; Netto *et al.* 2014; Prasher and Singh 2014; Chen *et al.* 2015; Linaldeddu *et al.* 2015; Trakunyingcharoen *et al.* 2015). It has also become clear that some of the reports of *L. theobromae* prior to 2004 represent other species of *Lasiodiplodia* and a new list of host species for this fungus is required.

Lasiodiplodia plurivora was the first cryptic species to be described in *Lasiodiplodia* (Damm *et al.* 2007), based on sequence variation in the internal transcribed spacer of the rDNA (ITS) and translation elongation factor-1α (*tef*1-α) regions. Shortly thereafter Alves *et al.* (2008) described *Lasiodiplodia parva* and *Lasiodiplodia pseudotheobromae* using the same loci. Subsequently, 20 additional species have been described in the *L. theobromae* complex. The majority of the 24 species that are now known in this complex cannot be identified based on morphology alone. Five species consistently group outside the *L. theobromae* species complex, namely *Lasiodiplodia crassispora*, *Lasiodiplodia gonubiensis*, *Lasiodiplodia pyriformis*, *Lasiodiplodia rubropurpurea*, and *Lasiodiplodia venezuelensis* (Pavlic *et al.* 2004; Burgess *et al.* 2006; Slippers *et al.* 2014).

The PSR concept provides the most powerful means to distinguish between taxa, also in terms of practical uses in quarantine and disease management. Unfortunately this approach is not without problems, especially where only a few loci are used. For example, hybridisation cannot always be recognised if sequences of only one (and often even two) loci have been considered. This is an important consideration because many fungi have the capacity to hybridize through sexual reproduction or exchange genetic material through anastomosis (fusion) of their vegetative hyphae in a parasexual cycle (Olson & Stenlid 2002; Schardl & Craven 2003; Stukenbrock 2016).

There are different possible outcomes of hybridisation in fungi, but only the two outcomes most applicable to this study will be discussed. The first and probably most common is introgression, where the hybrids in the population transfer novel genes to the parent population through backcrosses and the hybrid isolates eventually disappear from the population (Brasier 1995). The second outcome is the establishment of hybrid species that remain stable in the environment (Brasier 1995). These species are then described as *nothospecies* and indicated as hybrids with the symbol '×' as was done for *M. ×columbiana* (Newcombe *et al.* 2000), *Phytophthora ×alni*, *Phytophthora ×multiformis* (Husson *et al.* 2015), and *Phytophthora ×pelgrandis* (Nirenberg *et al.* 2009). It is important to indicate when a new species being described is a hybrid as these species can cause incongruence between different trees of different loci (Schardl & Craven 2003).

Lasiodiplodia occurs globally on woody plants in the tropics and sub-tropics (Punithalingam 1976). Species in the genus have been associated with many different plant diseases including fruit and root rots, die-back of branches and stem cankers (Burgess *et al.* 2006; Sakalidis *et al.* 2011a; Ismail *et al.* 2012; Urbez-Torres *et al.* 2012). *Lasiodiplodia* species have many different plant hosts, but pertinent to this study, they are also well-known on the iconic Baobab (*Adansonia* species), native to Africa and Australia (Roux 2002; Sakalidis *et al.* 2011a). In pathogenicity tests on the Australian baobab (*Adansonia gregorii*), *Lasiodiplodia iraniensis* and *Lasiodiplodia mahajangana* were shown to cause stem lesions and root rot (Sakalidis *et al.* 2011a).

The aims of this study were to identify species of *Lasiodiplodia* on baobab trees in Africa and to assess their ability to cause disease. We also evaluated the suitability of using sequence data from different nuclear loci for species delimitation in *Lasiodiplodia*. Using this information, all species in the genus were reassessed. The possible occurrence of hybrid *Lasiodiplodia* isolates from baobab trees, as well as in the previously described species was a specific focus.

Materials and methods

Sample collection and isolations

South Africa

Plant tissue samples from which to isolate endophytic *Botryosphaeriaceae* from baobab trees (*Adansonia digitata* s.l.) were collected during three surveys conducted in the Limpopo