FUNGAL BIOLOGY 121 (2017) 82–94

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio

Putative origins of the fungus *Leptographium procerum*

Stephen J. TAERUM[a,b,*], *Thierry B. HOAREAU*[c], *Tuan A. DUONG*[a], *Z. Wilhelm DE BEER*[d], *Robert JANKOWIAK*[e], *Michael J. WINGFIELD*[a]

[a]*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
[b]*China Eucalypt Research Centre, Chinese Academy of Forestry, Zhanjiang 524022, China*
[c]*Molecular Ecology and Evolution Programme, Department of Genetics, University of Pretoria, Pretoria 0002, South Africa*
[d]*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
[e]*Institute of Forest Ecosystem Protection, Department of Forest Pathology, Mycology and Tree Physiology, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Kraków, Poland*

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form
15 September 2016
Accepted 28 September 2016
Available online 5 October 2016
Corresponding Editor:
Kerry O'Donnell

Keywords:
Dendroctonus valens
Forestry
Invasion biology
Ophiostomatoid fungus
Symbiosis

ABSTRACT

Appropriate management of invasive fungi requires adequate understanding of their global diversities and movement histories. The fungus *Leptographium procerum* is associated with root-colonizing forest insects in pine forests throughout the world, and may have contributed to the aggressive behaviour of the red turpentine beetle (*Dendroctonus valens*) in the beetle's invasive range in China. We used microsatellites and mating type loci to investigate the global diversity of *L. procerum* and the source population of *L. procerum* associated with *D. valens* in China. Clustering analyses supported the separation of the fungal data set into three genetically and geographically-distinct clusters: Europe, North America, and China. The fungus had the highest genetic diversity in Europe, followed by North America and China. Analyses using Approximate Bayesian Computation supported Europe as the most likely source of the North American and Chinese populations. Overall, the results suggested that Europe is the global centre of diversity of *L. procerum*. Furthermore, they suggested that *L. procerum* most likely arrived in China independently of *D. valens* and adopted this beetle as a vector after its introduction.

Introduction

Rising global trade and travel combined with changing environmental conditions have resulted in an increased movement of fungi around the world (Palm 2001; Fisher & Garner 2007; Loo 2009). Invasive fungi have been understudied relative to invasive plants and animals, despite the considerable negative effects of some fungi on invaded environments (Gladieux *et al.* 2014). For example, the tree pathogens *Cryphonectria parasitica* and *Ophiostoma novo-ulmi* dramatically

---

* *Corresponding author*. Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private bag X20, Hatfield 0028, South Africa. Tel.: +27 124205821; fax: +27 124203960.
E-mail address: Stephen.Taerum@fabi.up.ac.za (S.J. Taerum).

http://dx.doi.org/10.1016/j.funbio.2016.09.007

changed forest structure in various parts of the world where they have killed large numbers of chestnuts and elms respectively (Loo 2009). In addition, the global spread of *Puccinia graminis*, a rust fungus that devastates cereal crops, has led to major crop losses in several locations (Palm 2001; Wanyera *et al.* 2006). More recently, the chytrid *Batrachochytrium dendrobatidis* has decimated amphibian populations around the world (Fisher & Garner 2007). Some fungi that are not pathogens in their native ranges have become problematic subsequent to their becoming invasive (Hulcr & Dunn 2011), suggesting that any exotic fungus is potentially damaging and therefore worth monitoring.

Ironically given their global relevance, the impacts of most invasive fungi are poorly understood because they are difficult to observe in the absence of substantial negative effects on the environment (Gladieux *et al.* 2014). Difficulties associated with conducting meaningful fungal surveys along with incorrect knowledge of whether a population of a pathogen is native or invasive can and has resulted in inaccurate conclusions regarding the movement histories of invasive pathogens, and by extension improper management strategies for the organisms (Estoup & Guillemaud 2010; Gladieux *et al.* 2014).

Accidental introductions of invasive fungi are often facilitated by animal vectors. Tree-colonizing insects are especially important vectors because several fungal species depend on these insects for dispersal as well as access to new host trees (Klepzig *et al.* 2009). If a species of tree-colonizing insect undergoes a range expansion or is introduced to a non-contiguous habitat, it can vector its fungal symbionts to environments in which they did not previously occur. In addition, introduced fungi sometimes undergo vector and host shifts, resulting in new associations between tree hosts, insect vectors, and fungal symbionts (Wingfield *et al.* 2010).

Invasions by fungal associates combined with vector and host shifts can have unpredictable, and sometimes serious, impacts on forest health (Ploetz *et al.* 2013; Wingfield *et al.* 2016). For example, some fungi have strong effects on the fitness of damaging tree-colonizing insects (Klepzig *et al.* 2009). Some phoretic fungi are mutualists of their insect associates (e.g., nutritional mutualists; Mueller *et al.* 2005), while others are antagonists (e.g., fungi that compete for substrates with their insect vectors; Cardoza *et al.* 2006). If the fungi are vectored by especially aggressive forest insects, the abundance of these fungi can greatly influence the frequency and intensity of insect outbreaks (Raffa *et al.* 2008). In addition, some phoretic fungi are serious pathogens of trees (Hulcr & Dunn 2011), in some cases causing more damage than their insect vectors.

The symbiosis between the fungus *Leptographium procerum* (Ophiostomatales, Ascomycota) and its vectors provides a model to study the global movement of an invasive fungus, as well as the interactions between invasive fungi, tree-colonizing insects, and invaded environments. *Leptographium procerum* is an ophiostomatoid (defined by Wingfield *et al.* 1993) fungus associated with various bark beetles and weevils (Coleoptera: Scolytinae) that typically infest conifers and especially the roots of *Pinus* spp (Jacobs & Wingfield 2001; Jankowiak *et al.* 2012). The fungus is also vectored by some cerambycid beetles (Coleoptera: Cerambycidae) that feed on roots and stumps of *Pinus sylvestris* (Jankowiak & Rossa 2007; Jankowiak 2010). The fungus has a frequently-observed asexual stage (Jacobs & Wingfield 2001), and is assumed to have a sexual stage based on the presence of mating type genes (Duong *et al.* 2013). Based on a recent study by Duong *et al.* (2013), *L. procerum* is a heterothallic fungus, meaning that each fungal culture possesses the genes for only one mating type, and must interact with individuals possessing the other mating type for meiosis to occur.

Leptographium procerum is frequently associated with the red turpentine beetle (*Dendroctonus valens*) in parts of the native range of the bark beetle in eastern North America (Taerum *et al.* 2013). The fungus appears to be a commensalist of the beetle, as the presence of *L. procerum* does not impact the development of *D. valens* larvae (Wang *et al.* 2012). Both the beetle and fungus are considered to be minor nuisances in North America, as the beetle primarily colonizes stressed or dying trees (Owen *et al.* 2010), while most pathogenicity trials have demonstrated that *L. procerum* gives rise to only small lesions on trees (Harrington 1988; Wingfield *et al.* 1988; Jankowiak *et al.* 2007; however, see Alexander *et al.* 1988).

Both *D. valens* and *L. procerum* appear to be more aggressive in their ranges in China. The beetle was accidentally introduced to China in the 1980's (Yan *et al.* 2005). *Leptographium procerum* was previously believed to have coinvaded China with *D. valens*, based on the fact that the fungus is associated with the beetle in its native range in eastern North America (Taerum *et al.* 2013), and that it has been reported in China as a symbiont of only this beetle (Lu *et al.* 2009a,b). Since 1999, *D. valens* has killed millions of healthy native pine trees in central China (Yan *et al.* 2005). Interactions between *D. valens* and *L. procerum* have been suggested to contribute to the tree-killing behaviour of the beetle as some *L. procerum* strains appear to be pathogenic to pines in China (Lu *et al.* 2010, 2011). In addition, there is evidence that trees infected with pathogenic strains of *L. procerum* in China may produce significantly larger quantities of the monoterpene 3-carene relative to healthy trees (Lu *et al.* 2010, 2011). This monoterpene is highly attractive to *D. valens*, and has been hypothesized to contribute to the tree-killing behaviour of the insect in China (Sun *et al.* 2013). This increased pathogenicity in China along with the production of more 3-carene could be due to rapid evolution of the fungus (Lu *et al.* 2011), although it could also be due to plasticity in the fungus or interactions with more susceptible hosts in China (Gladieux *et al.* 2014). Because *L. procerum* is the most common associate of *D. valens* in China (Lu *et al.* 2009a,b), it has been suggested that the fungus had a substantial impact on the outbreak dynamics of the bark beetle.

Although *L. procerum* has only been implicated as invasive in China (Lu *et al.* 2011; Sun *et al.* 2013; Taerum *et al.* 2013), it is present in many other locations around the world (Jacobs & Wingfield 2001). *Leptographium procerum* was originally described in eastern North America as an associate of several bark beetles and weevils (Kendrick 1962), and thus believed to be native to that continent. Later, the fungus was reported as an associate of various bark beetle and weevil species in England (Wingfield & Gibbs 1991), France (Piou 1993), Italy, Norway, the former Yugoslavia (Jacobs & Wingfield 2001), Poland (Jankowiak & Bilański 2013a,b,c), Russia (Linnakoski *et al.* 2012), Japan (Masuya *et al.* 2013), and New Zealand (Reay *et al.* 2005). Most of the studies in Europe and the study in Japan