

PERMANENT GENETIC RESOURCES NOTE

Characterization of microsatellite loci in the fungus, *Grosmannia clavigera*, a pine pathogen associated with the mountain pine beetle

C. K. M. TSUI,* N. FEAU,*† C. E. RITLAND,* S. MASSOUMI ALAMOUTI,‡ S. DiGUISTINI,‡
L. KHADEMPOUR,‡ J. BOHLMANN,§ C. BREUIL‡ and R. C. HAMELIN*†

*Department of Forest Science, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4, †Natural Resources Canada, 1055 rue du P.E.P.S., Ste-Foy, QC, Canada G1V 4C7, ‡Department of Wood Science, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4, §Michael Smith Laboratories, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Abstract

The largest forest pest epidemic in Canadian history caused by the mountain pine beetle (MPB) and its fungal associates has killed over 15 million hectares of forest. Sixty simple sequence repeat regions were identified from *Grosmannia clavigera*, an MPB associated fungus. Eight loci genotyped in 53 isolates from two populations in British Columbia, Canada revealed three to 10 alleles per locus and gene diversities of 0 to 0.79. All but two of these loci showed length polymorphism in *Leptographium longiclavatum*, a related MPB fungal associate. These microsatellites will be useful in population genetic studies of these fungi.

Keywords: genotyping, *Leptographium*, *Ophiostoma*, Ophiostomatales, population structure

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Western Canada is currently undergoing the largest mountain pine beetle (MPB) epidemic in recorded history, with over 15 million hectares of pine forests attacked (Kurz *et al.* 2008). MPB is closely associated with several fungi. One of the fungal associates, *Grosmannia clavigera* (Rob.-Jeffer. & R.W. Davidson) Zipfel, Z.W. Beer & M.J. Wingf. (Ophiostomatales, Ascomycetes), is pathogenic to pines and is believed to be an important component of the MPB epidemic (Lee *et al.* 2006). *Grosmannia clavigera* (formerly *Ophiostoma clavigerum*) produces asexual and sexual spores in a slimy mass that can be dispersed by beetles. It is haploid throughout most of its life cycle, but is transiently diploid when it reproduces sexually. The fungus grows rapidly in the host tree phloem and through the sapwood, producing melanin that discolours the wood (Paine *et al.* 1997). The mycelium blocks the host tree's water transport system eventually killing the tree (Yamaoka *et al.* 1995). Amplified fragment length polymorphism (AFLP) analyses showed that populations from the current epidemic were geneti-

cally variable (Lee *et al.* 2007). Polymorphic microsatellites would be valuable tools to supplement AFLP and improve our understanding of the population structure and migration patterns of the fungal associates of MPB. Thus, the aim of this study was to characterize such polymorphic microsatellite markers.

Simple sequence repeats (SSR) were identified from two resources: 5974 expressed sequence tags (ESTs) (DiGuistini *et al.* 2007) using SCIROKO (Kofler *et al.* 2007) and the draft genome of *G. clavigera* (DiGuistini *et al.* unpublished data) using Satelize (written in Python available upon request). Primers for polymerase chain reaction (PCR) amplification were designed from sequences flanking the microsatellites using Primer 3 (Rozen & Skaletsky 2000).

Sixty potential SSR were assessed including 43 and 17 SSR from EST and genomic resources respectively. Primer pairs were initially screened on DNA from 10 isolates pooled in equal amounts, amplified and analysed on silver-stained polyacrylamide gels (SequaGel[®] XE; National Diagnostic Inc.) for length polymorphism. The isolates included the holotype, as well as historical and genetically variable isolates (Lee *et al.* 2006, 2007). Genomic DNA was extracted from haploid mycelium

MPB. *Leptographium longiclavatum* is similar to *G. clavigera* in morphological features, and they are closely related in evolution (Lee *et al.* 2005). Two loci (SR45, SR47) showed considerable length polymorphism, and they will be useful for population genetic analysis of *L. longiclavatum*. The eight polymorphic microsatellite markers presented here will be valuable for future investigations of the population structure of *G. clavigera*.

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