SHORT, NON-REFEREED PAPER

BEAUVERIA BRONGNIARTII FUNGUS INFECTING WHITE GRUBS ATTACKING SUGARCANE IN THE KWAZULU-NATAL MIDLANDS NORTH REGION

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Abstract

Beauveria brongniartii (Saccardo) Petch epizootics were recorded at two sites in the Dalton area of the Midlands in South Africa on the melolonthid species, *Hypopholis sommeri* Burmeister (Coleoptera: Scarabaeidae). To identify the disease-causing fungus, 17 different fluorescently-labelled microsatellite PCR primers were used to target 78 isolates of *Beauveria* spp. DNA. Microsatellite data resolved two distinct clusters of *Beauveria* isolates which represented the *B. bassiana s.s.* (Balsamo) Vuillemin and *B. brongniartii* species groups. Groupings were supported by two gene regions, the nuclear ribosomal ITS and Bloc, of which 38 exemplar *Beauveria* isolates were represented and sequenced. Microsatellite analyses also showed that *B. brongniartii* conidia were being cycled from epigeal to subterranean habitats and *vice versa* in the environment by *H. sommeri* beetles. This is the first record of this species of fungus causing epizootics on melolonthid larvae and adults of *H. sommeri* in South Africa.

Keywords: biological control, genetic diversity, phylogeny, Scarabaeidae

Introduction

Since the early 1970s in the KwaZulu-Natal Midlands North region, sugarcane had been grown on land that was formerly under black wattle, *Acacia mearnsii* De Wild, cultivation (Carnegie, 1974). A known wattle chafer pest, *Hypopholis sommeri*, has now become damaging to sugarcane in this area (Carnegie, 1974). The increased incidence of this pest in the region has given rise to a number of observed natural fungal epizootics. The first epizootics in this region were observed on Sunnyside Farm in 2005, when hundreds of mycosed white grub cadavers were dug up from sugarcane fields. In 2010, two more farms only 10 km away, namely Harden Heights and Canema, were found to harbour fungal epizootics. At first it was suspected that *Beauveria bassiana* (Balsamo) Vuillemin was responsible for the death of white grubs however upon molecular investigation it was revealed that it was *Beauveria brongniartii* (Saccardo) Petch causing the observed epizootics on *H. sommeri* in this region.

Methods and Materials

Collection of isolates

Seventy-eight *Beauveria* isolates were collected from Harden Heights and Canema farms from infected scarabaeid larvae, adults (at the field edge) and pupae, and from the greater wax moth, *Galleria mellonella* (Zimmermann, 1986) and various plant surfaces (Meyling and Eilenberg, 2006). Isolates from other sites in South Africa as well as an isolate from Reunion Island (Bb1319-BT126) were also included.

Microsatellite DNA amplification and fragment analysis

Seventeen fluorescently-labelled microsatellite PCR primers obtained from Enkerli *et al*. (2001), Rehner and Buckley (2003) and Meyling *et al*. (2009) were used to target the *Beauveria* spp. DNA. PCR amplification reactions, their dilution and fragment analysis were performed according to Meyling *et al*. (2009). Resulting fragment analysis was undertaken using GeneMapper™ 3.1. A neighbour-joining analysis (30 000 bootstrap replications), using DARwin™ 5.0 (Perrier and Jacquemoud-Collet, 2006) was undertaken.

DNA extraction, PCR amplification, sequencing and phylogenetic analyses

Genomic DNA was extracted from *Beauveria* spp. isolates according to methods by Bayraktar *et al*. (2008). Twenty-three *Beauveria* isolates were partially amplified and sequenced for two nuclear loci: Bloc (intergenic region) and the ribosomal internal transcribed spacer (ITS). Also included were 15 published Bloc and ITS sequences from type-strain *Beauveria* species, as well as one outgroup isolate, *Isaria tenuipes*, in the phylogenetic analyses (Rehner *et al*. 2011). The Bloc and ITS gene regions were amplified and sequenced according to Rehner *et al*. (2006) and Rehner and Buckley (2005) respectively. Unrefined sequence chromatograms were assembled and edited with Geneious™ 5.4 Pro (Drummond *et al*. 2011). Multiple sequence alignments were created with MAFFT (Katoh *et al*. 2005; Katoh and Toh, 2008) using the FFT-NS-i alignment option. To determine the selection of nucleotide substitution model, implementing the Akaike information criterion for maximum likelihood (ML) and Bayesian (BI) analyses, jModelTest 0.1.1 (Posada, 2008) was used. The two-gene data set was partitioned. The substitution models for each partition were as follows: Bloc (TVM+G) and ITS (TIM2+G) and the combined data set (GTR+G). Maximum parsimony (MP), ML and BI analyses and their parameters were undertaken according to Rehner and Buckley (2005).

Results and Discussion

Microsatellite (SSR) analyses revealed two distinct *Beauveria* groupings supported by high bootstrap values (Figure 1). Of the 78 *Beauveria* isolates targeted by 17 microsatellite primers, 60 isolates (77%) fell into one closely-related SSR group 1, while 17 isolates grouped within the smaller, distantly-related SSR group 2.

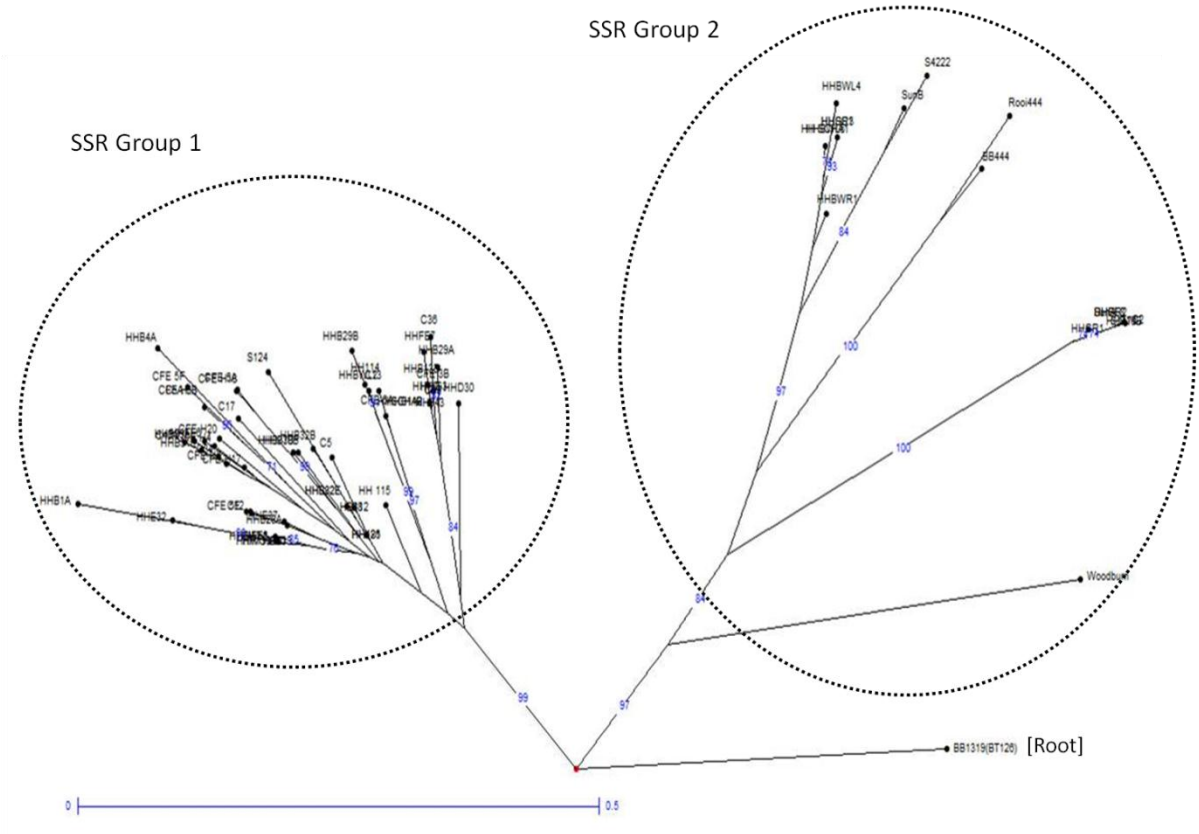

Figure 1. Unweighted, neighbour-joining tree in axial view, rooted on an isolate obtained from Reunion Island (BB1319-BT126), showing two distinct groupings of *Beauveria* isolates obtained from two sites in the KwaZulu-Natal Midlands North. Bootstrap values >70% are shown.

All three phylogenetic analyses (MP, ML and BI) yielded trees with similar tree topologies that each resolved eight terminal branches. Only four of these lineages were well-supported (Figure 2). Eight of the isolates from SSR group 2, fell within the common *B. bassiana s.s* clade A in the phylogeny (Figure 2). As previously reported, clade A represents a highly diverse species complex. Results showed that internal branch lengths in this clade were deep and internal groupings were observed, despite sampling localities being close together for some isolates (Figure 1). While *B. bassiana* isolates in this clade are capable of infecting white grubs, they are not responsible for the observed epizootics as only 7% were found on white grubs.

Fifteen of the SA isolates, which fell into the larger SSR group 1 (Figure 1) grouped into the *B. brongniartii* clade and were very closely related, as observed by the extremely short branch lengths (Figure 2). *B. brongniartii* isolates in this study appear to be genetically distinct from those found in the northern hemisphere, as seen by the separate grouping of the ARSEF isolates, although bootstrap and posterior probability values were not well supported at this node. *Beauveria australis* and *B. asiatica* were sisters to *B. brongniartii* in this study and received good bootstrap and posterior probability support, confirming the accuracy of the analyses and placement of the isolates. As described by previous studies, *B. caledonica* and *B. vermiconia* formed a sister pair with well-supported bootstrap and posterior probability support in this study. Sister to this group was *B. sungii* with no support. Unfortunately, due to poor sample inclusion, an accurate placement of the remaining basal *Beauveria* taxa, *B.*

pseudobassiana and *B. amorpha*, was not observed. Another commonality between this study and previous studies was the placement of *B. malawiensis* as the basal-most taxon (Figure 2).

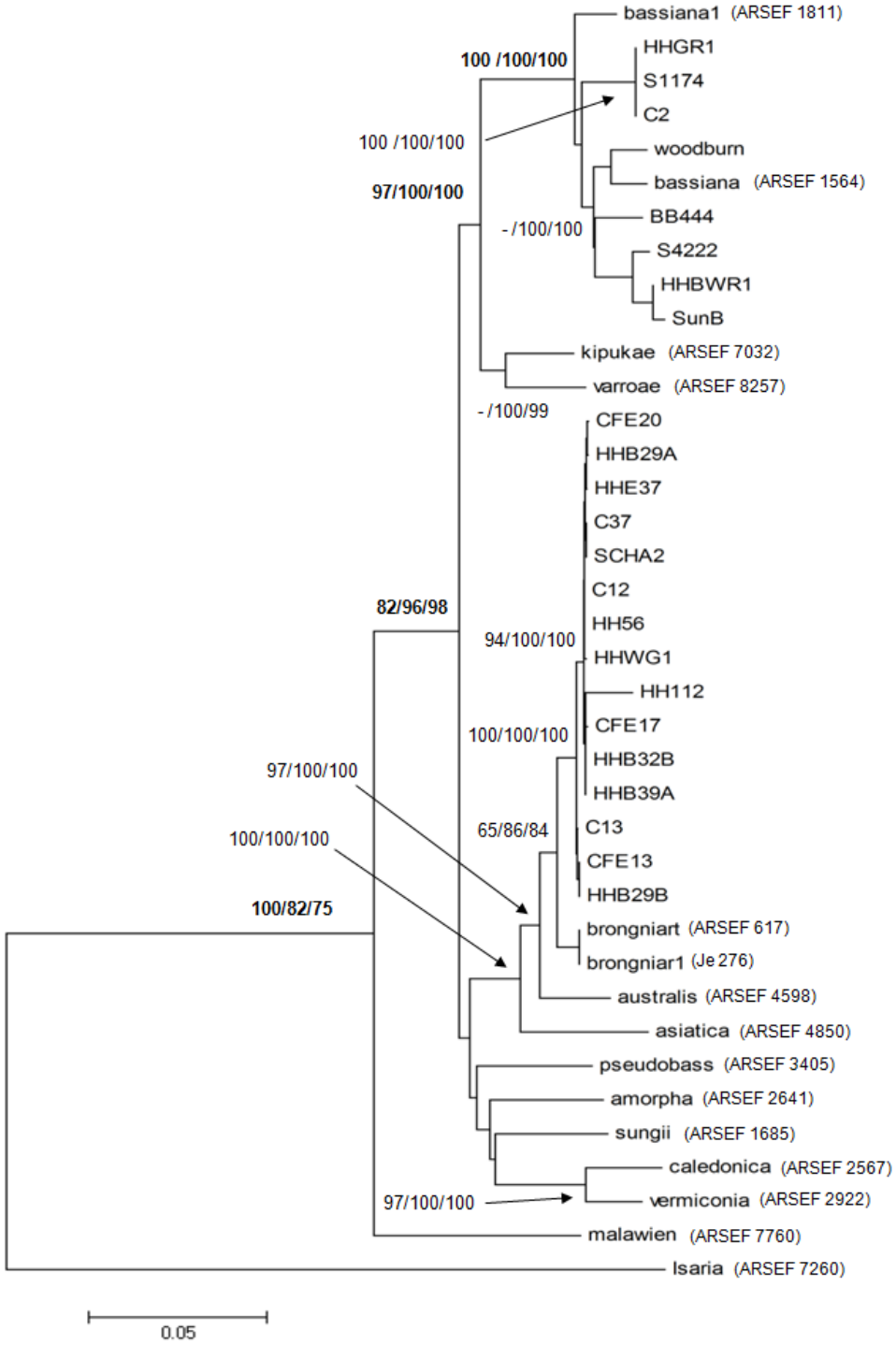

Figure 2. Phylogeny of *Beauveria* showing species relationships of South African isolates within the global *Beauveria* framework inferred from joint Bayesian Inference (BI) analysis of Bloc and ITS. The bootstrap values above internal branches correspond to Maximum Parsimony (>65), ML (>65) and Bayesian Inference posterior probabilities (>95) respectively.

When sequence data (Bloc and ITS) for three isolates (HHFE3, HH56 and HHBWL1) were considered, the isolates were found to be genetically identical (data not shown). Isolate HHFE3 was retrieved from an infected *H. sommeri* beetle found in a black wattle stand

adjacent to the Harden Heights sugarcane field. The same strain (HH56) was isolated from an infected *H. sommeri* L3 larva in the same sugarcane field. Finally, the same strain was again isolated from the leaf surface of a black wattle tree (HHBWL1) in the stand adjacent to the field. This indicates that this particular *B. brongniartii* strain was cycled from the epigeal (tree) environment to the subterranean environment and/or *vice versa*. This suggests that *H. sommeri* beetles may have a big role to play in the dissemination of the fungus for biological control of this insect pest species.

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