Extreme homozygosity in Southern Hemisphere populations of *Deladenus siricidicola*, a biological control agent of *Sirex noctilio*

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Abstract

The woodwasp *Sirex noctilio,* together with its mutualistic fungal symbiont *Amylostereum areolatum*, is the most damaging invasive pest of *Pinus* spp. in the Southern Hemisphere. The nematode *Deladenus siricidicola* parasitizes *S. noctilio* larvae and is the most effective biological control agent against this woodwasp. Nothing is known regarding the genetic diversity of *D. siricidicola,* even though such knowledge would be invaluable in improving sustainable biological control programs. The aim of this study was to develop microsatellite markers to study the genetic diversity of *D siricidicola* populations. Microsatellite enrichment was performed using Fast Isolation by AFLP of Sequences Containing repeats (FIASCO) and fragments were then sequenced using 454 GS-FLX pyrosequencing. From the 1.2 megabases of sequence data, 166 microsatellite containing contigs were identified. Twenty-six primer pairs were designed using the web-based program Primer3 and screened for polymorphism in populations of the nematode from different sources in the Southern Hemisphere. Seventeen primers amplified microsatellite-containing loci of interest. No length polymorphism was present in any of the microsatellite repeats in these populations. Regions flanking the microsatellites also showed no polymorphism, except for one transition observed in an Argentinean strain for locus *Ds316*. Twelve of the loci showed polymorphism between the Southern Hemisphere and Canadian sources of *D. siricidicola*. The lack of diversity in Southern Hemisphere populations of *D. siricidicola* could affect the ability of this nematode to adapt to different environments and host types where it is used in biological control programs, and should thus be considered as a factor in future control strategies and research projects.

Keywords: *Deladenus* (=*Beddingia*) *siricidicola*, Sirex woodwasp, entomopathogenic nematode, microsatellites, nematode population genetics

1. Introduction

The nematode *Deladenus siricidicola* Bedding is the primary and most effective biological control agent against the invasive woodwasp *Sirex noctilio*. This wasp and its obligate fungal mutualist, *Amylostereum areolatum* Boidin, is one of the most serious pests of *Pinus* spp. in forestry plantations in the Southern Hemisphere. *Deladenus siricidicola* has a bi-cyclic life cycle (Bedding, 1967; Bedding, 1972; Bedding and Akhurst, 1978). During the free-living cycle, the nematode feeds on the basidiomycete fungus *A. areolatum* growing in the infested trees. In its parasitic cycle, the nematode penetrates and develops inside the wasp larvae. Eggs of the nematode are triggered to convert to the infective form by the high CO_2 and low pH conditions that surround the Sirex larvae (Bedding, 1967; Bedding, 1972; Bedding and Iede, 2005). Once wasp pupation begins, the fertilized female nematodes release juveniles that migrate and infest the reproductive organs, eventually entering the eggs and sterilizing the female *S. noctilio*.

Sirex noctilio is native to Eurasia and North Africa (Morgan, 1968; Spradbery and Kirk, 1978) and was accidentally introduced into the Southern Hemisphere via New Zealand in the early 1900's. Subsequent biological invasions were observed in Australia in 1952 (Neumann et al., 1987), Uruguay in 1980 (Maderni, 1998), Argentina in 1985 (Klasmer et al., 1998), Brazil in 1988 (Iede et al., 1998), South Africa in 1994 (Tribe, 1995), Chile in 2000 (Ahumada, 2002) and more recently the United States of America in 2005 (Hoebeke et al., 2005) and Canada (de Groot et al., 2006).

Deladenus siricidicola was discovered in 1962 in New Zealand, parasitizing *S. noctilio* (Zondag, 1969). An extensive research program was established in New Zealand and Australia to characterize the biology of the nematode, and to develop a biological control program. This

program eventually resulted in the selection of a virulent Hungarian strain (Sopron strain) of *D. siricidicola* for use as a biocontrol agent (Bedding and Akhurst, 1974; Zondag, 1979). This strain was widely released in Sirex-infested plantations in Australia. In the late 1980's the Sopron strain was reported to have lost its virulence, presumably due to continuous mass rearing on the fungal symbiont (Haugen and Underdown, 1990, Bedding and Iede, 2005). A virulent strain of the nematode was then isolated from the Kamona forest (site of the original release of virulent Sopron strains) in Tasmania, and this Kamona strain was used to inoculate infested plantations in Australia (Bedding and Iede, 2005). The success of the program led to the distribution of the Kamona strain to other Sirex-infested countries in the Southern Hemisphere. A strain known as 'Encrusilada do sul' was isolated from the field-infested pines in Brazil and selected for biological control due to its higher level of virulence. The relationship between the Brazilian strain and Kamona has not been determined.

The rate of *D. siricidicola* parasitism of Sirex wasps in inoculated trees varies between 5 – 90 % in different regions of the Southern Hemisphere (Hurley et al., 2007). These authors hypothesized that a number of factors could affect this variation in parasitism by the nematode, including inoculation techniques, moisture content of the wood, loss of virulence of the nematode, incompatibility among the specific populations of *S. noctilio*, *A. areolatum* and *D. siricidicola* in that area, competition of saprophytic fungi with *A. areolatum*, and pine species affected. In a subsequent study, Hurley et al. (2008) excluded inoculation techniques and loss of nematode virulence as factors affecting *D. siricidicola* parasitism in South Africa. While wood moisture content affected parasitism levels, this did not explain the breadth of the observed variation. This highlights the possible role of other factors that might affect nematode efficacy and that remain to be tested.

Population diversity studies on *D. siricidicola* and *S. noctilio* have yet to be conducted. The aim of this study was to characterize microsatellite containing markers specific for *D. siricidicola* that could be used in studies of population genetic variation. These markers are then applied to assess the diversity among strains from populations of *D. siricidicola* from Australia, Argentina, Brazil and South Africa as well as to determine the level of diversity and the historical relationship between these nematode strains. This diversity is also compared with that of strains of the nematode that have recently been discovered in Canada (Yu et al., 2009).

2 Material and Methods

2.1 Nematode sources and harvesting

Sources of *D. siricidicola* were from Argentina (two sources; Argentina1, Argentina2), Australia, Brazil and South Africa for the Southern Hemisphere, and Canada from the Northern Hemisphere (four sources; 181, 184, 484 and 1089). Nematode sources refer to cultures of the nematode made from an individual wasp in the different regions of origin. All nematode cultures are maintained by the Tree Protection Co-operative Program based in the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (http://www.fabinet.up.ac.za).

Nematodes were harvested from cultures grown in 500 ml Erlenmeyer flasks containing wheat, rice and *Amylostereum areolatum*, or on potato dextrose agar (PDA) plates onto which *A. areolatum* had been inoculated (Bedding and Akhurst, 1974). Nematodes were harvested from the flasks by soaking (15 min.) and washing with distilled H_20. The nematode suspension was poured through a sieve into 1 L beakers and nematodes were allowed to settle for 20 min. For

isolation from Petri dish culture plates, sterile distilled water (SABAX water, Adcock Ingram Ltd, Bryanston, RSA) was added to the plates to cover the cultures and left to stand for 20 min, while swirling four times during this period. The suspension was decanted into 50 ml beakers and nematodes were allowed to settle for 10 min. After settling, for both of the above techniques, excess water was discarded and nematodes were washed four times at intervals of 20 min in sterile distilled water, each time discarding excess water, prior to the subsequent wash. Following the last wash, 1 ml of sample was transferred to 1.5 ml Eppendorf tubes, centrifuged at 13 000 rpm for 3 min, the supernatant was discarded and the sediment was used for DNA extraction.

2.2 DNA extraction

A phenol-chloroform DNA extraction method was used to extract DNA for microsatellite enrichment and discovery, from large numbers of harvested nematodes from flasks as described above (source Argentina1). The nucleic acids were precipitated by 0.1 volume of 2M NaAc (pH 5.6) and 1 ml of 100 % EtOH. The sample was left overnight and the DNA was obtained after centrifugation (Eppendorf Centrifuge 5417C), washing the pellet with 1 ml of 70 % EtOH, vacuum drying at 45°C for 5 min (Concentrator 5301), and resuspending with 100 µl of Sabax water. Five microlitres of RNase were added to the resuspended sample, followed by incubation on HB-2 heat blocks (Wealtec Corporation) at 37°C for 3 hours.

Pooled DNA extractions from all nematode sources were performed using PrepMan™ (Applied Biosystems, California, USA) in 20 µl, and using nematodes harvested from PDA culture plates as described above. Fifteen microlitres of PrepMan Ultra sample Preparation Reagent and a 2

mm-diameter sterile metal ball was added to each sample-containing Eppendorf tube. The samples were shaken at a frequency of 30 vibrations/s for 3 min using the Retsch® MM 301 (Retsch, Germany). The metal balls were then removed with a magnetic stick, and the samples vortexed for 15 s and incubated at 100 °C for 10 min. Following incubation, samples were centrifuged at 13 000 rpm for 3 min, after which the DNA containing supernatant was transferred into sterile 1.5 ml Eppendorf tubes. The concentration of the resulting DNA was determined using the ND – 1000 UV/Vis Spectrometer (NanoDrop Technologies, Wilmington, DE USA).

2.3 Microsatellite enrichment and pyrosequencing

The fast isolation by AFLP sequence containing repeats (FIASCO) method (Zane et al., 2002) with modifications (M-FIASCO) (Cortinas et al., 2006) was used for microsatellite enrichment of *D. siricidicola* source Argentina1. This was followed by pyrosequencing as described by Santana et al. (2009).

2.4 Primer design and amplification

Sequences obtained from pyrosequencing were assembled into contigs using the ContigExpress component of Vector NTI Explorer software (Invitrogen, Carlsbad, CA, USA). From these contigs, microsatellite repeats were identified using the web-based program Simple Sequence Repeat Identification Tool (SSRIT) (Temnykh et al., 2001). The minimum number of repeats used in order to design primers for microsatellite containing sequences was five repeats for all

repeat classes. Primers were designed using the PRIMER 3.0 software (Rozen and Skalestsky, 2000).

Primers were optimized and used on all *D. siricidicola* sources from harvested cultures (i.e. large numbers of nematodes pooled in each of the DNA isolation reactions). Polymerase Chain Reactions (PCR's) were performed using the thermal *i*Cycler (Bio-Rad). DNA from various populations was used as the template in a 25 µl reaction volume containing 10× PCR buffer (10x solution, 100 mM Tris-HCl, 500 mM KCl, pH 8.3) (Roche Diagnostics GmbH, Germany), 25mM $MgCl_2$ (Roche Diagnostics GmbH), 10 mM dNTPs, 10µM of each forward and reverse primer, FastStart *Taq* DNA Polymerase (Roche Diagnostics GmbH)and sterile SABAX water was added to make up the total volume for PCR amplification. The thermocycler conditions were an initial denaturation step at 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension step of 72 °C for 10 min. PCR products were separated on 2 % agarose gels, stained with ethidium bromide (EtBr) and visualized under UV transilluminator imaging system (UVP, United Kingdom). A 100 base-pair (bp) molecular weight marker (Fermentas, O' Gene Ruler ™) was used for size estimates of PCR products.

The presence of *A. areolatum* DNA in *D. siricidicola* extracted DNA was determined using basidiomycete primers, P – 1 (5' TTG CAG ACG ACT TGA ATG G 3') (Hsiau, 1996) and 5S – 2B (5' CAC CGC ATC CCG TCT GAT CTG CG 3') (Coetzee et al., 2000). These primers targeting the IGS rDNA region were also tested on *A. areolatum* isolate F8 NCT (*A. areolatum* isolate from the field populations of *S. noctilio* in South Africa) using the same conditions as for *D. siricidicola* DNA.

For Southern Hemisphere sources of *D. siricidicola,* the microsatellite loci (excluding Ds 105, Ds 302, Ds 323, Ds 350 and 366) were also amplified from four single nematodes from all sources. For single nematode amplification, nematodes were transferred from *A. areolatum* cultures using 0.80 x 40 mm sterile needles (New Promex Corporation) into 96 well PCR microplates (Axygen Scientific, California) using a Stemmi 2000 microscope (Zeiss, Germany). Each well contained one nematode in a 25 µl of the PCR reagent mixture as described above. The thermocycler conditions for amplification were an initial denaturation step at 95 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60°C for 30 s, extension at 72 °C for 1 min and a final extension step of 72°C for 10 min. The PCR products were visualized as described above. In order to assess possible polymorphism, PCR products from single nematodes were separated by 15 % polyacrylamide gel electrophoresis (PAGE) (30 % acrylamide, 10x Tris-borate-EDTA buffer, 10 % APS and TEMED; 4h at 160V). Once the run was complete, the gels were placed into a Syber Gold (Molecular Probes, Inc., Eugene, OR, USA) solution for 30 min and then visualized under the UV transilluminator.

2.5 DNA sequencing

PCR products were purified using 6 % Sephadex G-50 columns with 50 – 150 µm bead size (Sigma Aldrich, Germany) following manufacturer's instructions, before sequencing. Purified PCR products were used as template DNA in cycle sequence reactions using the ABI BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems). The same primers used for PCR amplification were used to sequence both strands during cycle sequencing reactions. The thermocycler conditions were 25 cycles of denaturation at 96 °C for 10 s, annealing at 55 °C for

15 s and extension at 60 °C for 4 min using the thermal *i*Cycler (Bio-Rad). The products were purified as previously described and precipitated using the Concentrator 5301 (Eppendorf AG, Germany) at 45 °C for 30 min. Precipitated PCR products were run on an ABI PRISM 377 automated DNA sequencer (Applied Biosystems, California, USA).

3. Results

Both harvesting methods yielded large numbers of nematodes from which DNA was extracted. DNA extraction from the flask-reared nematodes using the phenol – chloroform method was considerably more labor intensive and time consuming compared to DNA extraction performed using PrepMan™ (Applied Biosystems, California, USA) from PDA cultures.

The outcomes of the FIASCO enrichment and pyrosequencing have previously been reported and compared with a similar application of these tools to the fungus *Fusarium circinatum* and *Sirex noctilio* by Santana et al. (2009). In summary, 1.2 Mb of sequence data were generated using this method, which was assembled into 397 contigs or fragments represented by single reads. Of these, 166 fragments contained microsatellites with dinucleotides, trinucleotides and tetranucleotides, comprising 93 %, 2 % and 5 % of the fragments, respectively.

A total of 26 primer pairs were designed to amplify microsatellite-containing loci, of which 17 could be optimized to amplify single fragments (Table 1). These fragments were confirmed to represent the loci of interest through sequencing and comparison with the original fragments. Amplifications were equally successful from DNA extracted from culture plates (including large numbers of nematodes per sample) and for amplifications from single nematodes. DNA of *A. areolatum* was detected in the extracted nematode DNA through amplification of the IGS rDNA

region using the primers P-1 and 5S-2B. None of the primer pairs resulted in the amplification of fragments when DNA extracted from *A. areolatum* cultures was used in the reactions.

No length polymorphisms were identified in any of the 17 microsatellite containing loci using agarose and polyacrylamide gel electrophoresis for any of the five sources of *D. siricidicola* from the Southern Hemisphere. This was confirmed by sequencing 10 - 25 representative products for each of the 12 loci. These sequences also confirmed that there were no polymorphisms in regions flanking the microsatellites, except for one transition (T/C) observed for the strain Argentina2 for locus *Ds316*, compared to other sources of the nematode from the Southern Hemisphere. The sequence data for 17 primer pairs covered 3291 bp including 317 bp of microsatellite repeated sequences.

Comparison of PCR product sequences for the 17 selected loci between Southern Hemisphere and Canadian sources of the nematode (DNA isolated from culture plates) revealed polymorphisms in 12 of the loci (Table 1 and 2). These polymorphisms included microsatellite length variations, single nucleotide polymorphisms and insertions/deletions (indels) (Table 2).

4. Discussion

This study is the first to consider the diversity among populations of *D. siricidicola* from different regions in the Southern Hemisphere. It was expected that there might be reduced diversity and a genetic linkage between these populations, given their shared history of introduction and the expected bottlenecks resulting from the rearing process. The extent of homozygosity was, however, unexpected. Among 3291 bp of sequence data, spanning 317 bp of microsatellite repeats, there was only one base pair variation between a source obtained from

Argentina and all other sources of the nematode from the Southern Hemisphere. These results confirm that the Kamona strain has been distributed throughout the Southern Hemisphere, and from a single origin. In contrast, strains of *D. siricidicola* from Canada provided a firm basis for comparison and were clearly genetically distinct from all the Southern Hemisphere strains.

The analysis of the Southern Hemisphere populations of *D. siricidicola* using pooled DNA from cultures, confirmed by genotyping single nematodes from all populations, showed that a highly inbred population of the nematode exists throughout the Southern Hemisphere. This is likely the result of repeated genetic bottlenecks and inbreeding associated with the culturing and introduction of the nematode into new environments. When nematodes are reared, a circular plug of 13 mm in diameter containing between >100 – >2000 nematodes (Brett Hurley, unpublished data) is selected from a culture, which is then used to repeatedly produce subcultures. The plug is also likely to contain many nematode siblings, since female nematodes lay their eggs in groups. Such sub-culturing typically occurs bi-weekly, until the nematodes are transferred onto flasks for mass production or stored in liquid nitrogen (Bedding and Iede, 2005). When *D. siricidicola* is introduced into new regions for biological control programs, only a few cultures are typically introduced. These steps of multiplication, bulking and transfer to new environments, repeated in many laboratories during the course of the last two decades, will inevitably have resulted in genetic bottlenecks and inbreeding for the Kamona strain of *D. siricidicola*. Results of this study confirm this view.

Founder effects and inbreeding have been shown to affect the population diversity of other nematodes in a similar fashion to *D. siricidicola*. For example, a study conducted on the pine wood nematode, *Bursaphelenchus xylophilus*, an introduced pathogen in Japan (Mamiya, 1987), showed a low degree of microsatellite polymorphism (relative genetic uniformity) in sub-

populations (Zhou et al., 2007). This was attributed to a founder effect. Similarly, studies on the cyst nematodes *Heterodera schachtti* (Plantard and Porte, 2003; Plantard and Porte, 2004) and *Globodera pallida* (Picard et al., 2004) have shown that they are highly inbred species due to increased probability of interbreeding between siblings mating, which is influenced by their limited dispersal range. Larvae from a single cyst are siblings or half-siblings, depending on whether a single or several males fertilize a female. This mode of reproduction can significantly influence and reduce population diversity. The same situation appears to be true for *D. siricidicola* in the Southern Hemisphere.

Comparison of *D. siricidicola* sources from the Southern Hemisphere and Canada confirmed that each of these areas harbours a different strain of the nematode. *Deladenus siricidicola* was thought not to be present in North America, and was only recently discovered in that region (Bedding and Akhurst, 1978; Yu et al., 2009). Yu et al. (2009) confirmed the species identity using morphological characteristics and comparison with the Kamona strain based on DNA sequences. Using sequences for ribosomal DNA region and the cytochrome oxidase subunit 1 (COI), these authors also showed that there were sequence differences between North America *D. siricidicola* and the Kamona strain. These differences included seven substitutions and ten indels for the rDNA region, and two base pair differences for the COI. However, subsequent work has shown that a portion of the Canadian population is the same or similar to the Kamona strain (Isabel Leal, unpublished data). The microsatellite markers developed here will be useful to further characterize the differences between the Canadian and Kamona strain of *D. siricidicola*.

It is commonly accepted that *D. siricidicola* was accidentally introduced into Canada. Such an introduction would not be unusual, in view of the fact that the original discovery of the nematode

in New Zealand during the early part of the 20th century resulted from its accidental introduction together with *S. noctilio* (Zondag, 1969). In tracing the origin of the *S. noctilio* invasion into the United States of America, Nielsen et al. (2009) showed that *A. areolatum* found in North America was represented by two genotypes, which were different from the genotypes found in the Southern Hemisphere (Slippers et al., 2001; Slippers et al., 2002). These results suggest that the invasions of *S. noctilio* into the Southern Hemisphere and North America represent two separate events. In contrast, a recent study using multiple locus sequence data has suggested that one of the genotypes of *A. areolatum* in Canada is identical to that in the Southern Hemisphere (Bergeron et al., 2011). This would imply a shared origin of introduction of *S. noctilio* into North America and the Southern Hemisphere. Regardless of whether there is a link between the *S. noctilio* introductions in North America and the Southern Hemisphere, the introduction of *D. siricidicola* appears not to be connected to that of the fungal strains observed in the Southern Hemisphere. This provides an opportunity for North American and Southern Hemisphere countries to exchange nematode strains and thus to increase genetic diversity and potentially the efficacy of biological control programs in these regions.

The loci for which markers have been developed in this study might have broader application than simply microsatellite length variation in populations of *D. siricidicola*. A number of loci also contained SNPs and indels in the microsatellite flanking regions. These variations were in some cases fixed in the populations, for example between those of the Kamona strain from the Southern Hemisphere and strains from Canada. These markers could thus be used for rapid distinction between the strains using sequencing, real-time PCR probes or primers, or PCR-RFLP screens (Nasmith et al., 1996; Aikawa et al., 2006).

Next generation sequencing coupled to microsatellite enrichment protocols has provided important new opportunities for the effective development of microsatellite markers for population studies, as illustrated in this study. The use of pyrosequencing enabled the exclusion of the time–consuming cloning step, generated substantially more microsatellite containing sequences than would have been feasible with the traditional cloning and Sanger sequencing approaches, and substantially reduced the overall costs associated with development of microsatellites. This tool has recently been described in a series of studies, with slight modifications, and applied widely to various organisms. These include the venomous copperhead snake *Agkistrodon contortrix* (Castoe et al., 2010), the pine pathogenic fungus *Fusarium circinatum*, the pine-damaging woodwasp *Sirex noctilio* and its parasitic nematode *Deladenus siricidicola* (Santana et al., 2009), ancient DNA of the extinct heavy-footed moa *Pachyornis elephantopus* (Allentoft et al., 2009), and New Zealand's endangered blue duck *Hymenolaimus malacorhynchos* (Abdelkrim et al., 2009). Interestingly, in the latter two studies, microsatellites are known to occur in low frequency, and yet this tool enabled characterization of markers that are crucial in understanding past biodiversity and extinction processes.

The nematode harvesting method used in this study did not rid the *D. siricidicola* samples of all residual *A. areolatum* fragments, even after multiple washes. This meant that DNA extracts contained a mixture of fungal and nematode DNA, and therefore a possibility of inadvertently developing primers from fungal sequences. All primers were therefore tested for their ability to amplify pure *A. areolatum* DNA, but this did not occur. This is probably because the fungal DNA was in a much lower concentration compared to the nematode DNA. The primers can thus now be applied in situations in which even greater amounts of fungal DNA are present.

It is of substantial concern that Southern Hemisphere populations of *D. siricidicola* appear to be highly inbred, and also homozygous, at most of the loci tested. This lack of diversity is likely to significantly reduce the ability of the nematode to adapt and establish itself in different environments, on different *Pinus* spp. and on different populations of *S. noctilio* (Roderick and Navajas, 2003; Hufbauer and Roderick, 2005). The environments in which the nematode is applied for biological control purposes include a variety of climate types, from Mediterranean winter-rainfall, to continental summer-rainfall, cold temperate winter rainfall and subtropical environments. In addition, various *Pinus* spp. are involved, including *P. radiata, P. patula, P. elliottii, P. sylvestris, P. taeda,* and *P. caribaea, P. contorta var. latifolia* and *P. ponderosa*. It is also known that the fungus *A. areolatum*, which is an obligate food source for the nematode, and different wasp populations can differ genetically and phenotypically across regions (Hurley et al., 2007; Hurley et al., 2008). These authors also highlight the fact that there are other genera of fungi in trees where the nematode is applied, which also have the potential to reduce the efficacy of *D. siricidicola* as a biocontrol agent. These and probably other less obvious factors make it highly unlikely that a genetically homozygous population of *D. siricidicola* will be an effective biocontrol agent over the longer term. Thus, for an effective biological control program for the Southern Hemisphere, native and genetically diverse populations of *D. siricidicola* would need to be characterized and tested in different environments where biological control is required. Apart from native environments, New Zealand might also be a location from which diverse strains of *D. siricidicola* could be obtained, or observed. This region has had many introductions of the nematode spanning a long period of time. The Kamona strain of *D. siricidicola* has also not been deployed in New Zealand, and therefore the nematode populations in this country might be sufficiently genetically diverse to provide new sources of the biocontrol agent. The

polymorphic markers produced in this study should be useful in future research requiring knowledge of the genetic diversity and structure of *D. siricidicola* populations.

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Table 1. Primer sequences, expected locus size per SSR primers, no. of SSR repeats and annealing temperature per primer of *Deladenus siricidicola*.

Locus	Primer name	Primer sequence (5' → 3')	Expected size	Repeats	Tm (°C)	Polymorphism (SH vs Can)
Ds 1	Ds 1F	CAATGTGCTGCGTCAATTTT	157 bp	$(GTA)_{10}$	56	Yes
	Ds 1R	ACCCAACGCGTAGTGATAGC			62	
Ds 7	Ds 7F	TTCAATTTTGTGTCTGGCAAA	249 bp	$(TG)_6$	55	No
	Ds 7R	AGCCACAAATCGCGACATA			58	
Ds 19	Ds 19F	CGTGACCGATTTCATTTTGC	192 bp	$(TCC)_5$	58	No
	Ds 19R	ACGACAGCGAAGAGAACGAT			60	
Ds 54	Ds 54F	CAGCCACAACAATTCACACC	155 bp	$(CA)_6$	60	Yes
	Ds 54R	GCACAAAAATCTCGCCTCAT			58	
Ds 83	Ds 83F	AGGCATAGAGCGAGTGGAAA	159 bp	$(GA)_5$	60	Yes
	Ds 83R	TCTCACGAACTTGTCCCTCA			59	
Ds 105	Ds 105F	TGGTAGCAATCGATCGAAAA	150 bp	$(AG)_9$	56	Yes
	Ds 105R	CGTGTCCACTTGTCCCTCTC			56	
Ds 201	Ds 201F	TGCATAGCTGGCGATAAATG	168 bp	$(TG)_7$	58	Yes
	Ds 201R	CGAGTCACGTACGCATTAGC			62	
Ds 302	Ds 302F	ATTGTTACGGTGTGGGCATT	215 bp	$(AC)_6$ $(CA)_5$	58	Yes
	Ds 302R	TGGATGTCCGTCTGTTGTGT			60	
Ds 308	Ds 308F	GCCTTTTCCTTAGCTGTTTGA	155 bp	$(TG)_6$	59	No
	Ds 308R	GTGGTCCTCCTCTCCCTTTT			62	
Ds 316	Ds 316F	TGCGGATATCTTCTCATTGTAA	382 bp	$(TC)_7$ $(TC)_5$	57	Yes
	Ds 316R	TCAAATGTTATGCGAAATTCTG			55	
Ds 318	Ds 318F	AGGGTACTCATGCCGAGGTT	127 bp	$(AC)_6$	63	No
	Ds 318R	ATGCGTATGTGCAGTTGTGT			58	
Ds 323	Ds 323F	TTTACCTGTTGGCTGTTACCG	165 bp	$(TG)_8$ $(TG)_6$	61	Yes
	Ds 323R	TGGGGTAAAAGTGGATTGGA			58	
Ds 325	Ds 325F	ACGCTTATGTGTGCCACTTG	298 bp	$(TG)_6$ $(GT)_8$ $(TG)_5$ $(CATA)_5$	60	Yes
	Ds 325R	GGGTCTCTTGATGATGTTTCG			61	

Ds 350	Ds 350F	AGTCCTGAGTAACCTCCACCA	151 bp	$(GC)_5$	63	No
	Ds 350R	TACGGTACGCTGATGCTCAC			62	
Ds 366	Ds 366F	CGCTGCTGTACTGCTGTTTT	152 bp	$(GT)_5$	60	Yes
	Ds 366R	CACACAAATGCACACATGGA			58	
Ds 375	Ds 375F	GGCAGCTGAAATGATGACAA	188 bp	$(AC)_5$	58	Yes
	Ds 375R	ATCATCATCAGCAATATCCTCA			57	
Ds 388	Ds 388F	AAGTCAGCTGAAAGGCGAAG	228 bp	$(CA)_{10}$ $(AC)_4$	60	Yes
	Ds 388R	TGTGTGCATGAAAACGGAAC			58	

SH – Southern Hemisphere sources (Argentina1, Argentina2, Australia, Brazil & South Africa)
Can - Canadian sources (181, 184, 484, 1084)

Table 2. Summary of polymorphisms observed per locus.

Primers	Strains	Types of polymorphism			No. of alleles
		SSRs	SNPs	Insertion	
Ds 1	SH	$(GTA)_{10}$	A/G	-	1
	Canadian	$(GTA)_{16}$		GTGGTAGT	1
Ds 54	SH	No variation	-	CACATACA	1
	Canadian	No variation		-	1
Ds 83	SH	No variation	G/T ; A/G	-	1
	Canadian	No variation		-	1
Ds 105	SH	$(AG)_9$	G/A	-	1
	Canadian	$(AG)_{13}$		-	1
Ds 201	SH	$(TG)_7$	G/A	T	1
	Canadian	$(TG)_4$		-	1
Ds 302	SH	No variation	-	-	1
	Canadian	No variation		T	1
Ds 316	SH	$(GA)_5$	-	-	1
	Canadian	$(GA)_7$		-	1
Ds 323	SH	$(TG)_8$	G/T	TTGT	1
	Canadian	$(TG)_7$		-	1
Ds 325	SH	$(GT)_8$	A/G ; A/T	-	1
	Canadian	$(GT)_9$		-	2
Ds 366	SH	$(GT)_5$	T/A	-	1
	Canadian	$(GT)_6$		-	1
Ds 375	SH	No variation	-	TGCACA	1
	Canadian	No variation		-	1
Ds 388	SH	$(GT)_4 (TG)_{10}$	C/T	-	1
	Canadian	$(GT)_5 (TG)_7$		-	1

SH – Southern Hemisphere sources (Argentina1, Argentina2, Australia, Brazil & South Africa)
Canadian - Canadian sources (181, 184, 484, 1084)