



## The global diversity of *Deladenus siricidicola* in native and non-native populations

Katrin N.E. Fitzg<sup>a</sup>, Jeff R. Garnas<sup>b,c</sup>, Maria J. Lombardero<sup>d</sup>, Matthew P. Ayres<sup>e</sup>,  
Flora E. Krivak-Tetley<sup>e</sup>, Rodrigo Ahumada<sup>f</sup>, Brett P. Hurley<sup>b</sup>, Michael J. Wingfield<sup>a</sup>,  
Bernard Slippers<sup>a,\*</sup>

<sup>a</sup> Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa

<sup>b</sup> Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa

<sup>c</sup> Department of Natural Resources and the Environment, University of New Hampshire, NH, USA

<sup>d</sup> Departamento de Producción Vegetal, Universidad de Santiago, 27002 Lugo, Spain

<sup>e</sup> Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

<sup>f</sup> Bioforest S.A., Casilla 70-C, Concepción, Chile

### ARTICLE INFO

#### Keywords:

Biological control  
*Deladenus siricidicola*  
*Sirex noctilio*  
Invasive pest  
Population genetics

### ABSTRACT

The nematode *Deladenus siricidicola* is the primary biological control agent of *Sirex noctilio*, a globally invading woodwasp pest of *Pinus* species. Preliminary studies on the diversity of populations of *D. siricidicola* revealed very low diversity in the Southern Hemisphere where they have been introduced for the purpose of biological control. The potential to augment biocontrol efficacy by increasing genetic diversity in biocontrol programs motivated this study, which investigated the patterns of genetic diversity in *D. siricidicola* across eight countries, including the presumed native range (Spain), areas of accidental introduction (Canada and the USA) and countries *D. siricidicola* has been intentionally released (Argentina, Brazil, Chile, New Zealand and South Africa). Nematodes were screened using mitochondrial COI sequence data and twelve microsatellite markers. Analyses of these data identified three distinct lineages from North America (Lineage A), the Southern Hemisphere (Lineage B) and Spain (Lineage C). Strains from Chile were an exception as they appear to represent an admixture of lineages A and B. This suggests a common origin of populations throughout the Southern Hemisphere, with a second introduction from North America into Chile. The introduction into North America is distinct from that in the Southern Hemisphere and probably originated from Europe. It is evident that substantial genetic diversity exists in *D. siricidicola* globally, which could be exploited to augment the reduced diversity in some populations used in biocontrol programs.

### 1. Introduction

Many biological control organisms are successful despite passing through a genetic bottleneck. There is also little concrete evidence that genetic diversity is necessary for biocontrol success (Holt and Hochberg, 1997; Stouthamer and Kazmer, 1994), although some examples exist (Hufbauer, 2002; Phillips et al., 2008; Tomasetto et al., 2017). However, conventional wisdom posits that genetic diversity generally has fitness advantages for populations (Roderick and Navajas, 2003), and there are many reasons to think that augmenting genetic diversity might be beneficial in biocontrol systems. Firstly, genetic diversity of biological control agents can impact establishment and efficiency (Forsman, 2014; Szűcs et al., 2017), as well as the potential for

co-evolution with hosts. Furthermore, genetic diversity can also positively affect adaptive potential to a range of climates (Booy et al., 2000), or the reproductive fitness of the biological control agent (Joron and Brakefield, 2003; Reed and Frankham, 2003). Additionally, diversity reduces risks of negative effects from inbreeding. On the negative side, high genetic diversity in biological control agents could also increase the probability of non-target effects via host-switching, though empirical examples of this relationship are lacking (Roderick and Navajas, 2003).

The diversity of biological control agents can be especially important when considering the complex patterns of invasion of the pests against which they are deployed (Garnas et al., 2016). Multiple introductions of invasive pests often result in increased genetic diversity

\* Corresponding author.

E-mail address: [Bernard.Slippers@fabi.up.ac.za](mailto:Bernard.Slippers@fabi.up.ac.za) (B. Slippers).

<https://doi.org/10.1016/j.biocontrol.2019.01.014>

Received 27 April 2018; Received in revised form 11 January 2019; Accepted 27 January 2019

Available online 28 January 2019

1049-9644/ © 2019 Elsevier Inc. All rights reserved.

and population substructure across the invasive range, which could complicate pest management efforts (Garnas et al., 2016). Such is the case for the target pest of the current study; the woodwasp *Sirex noctilio* that is an invasive pest of *Pinus* on four continents. Boissin et al. (2012) analysed the global diversity of *S. noctilio* populations and considered potential routes of invasion. The results suggested that multiple introductions occurred in various parts of the world, leading to a high degree of admixture.

The main biological control agent used for the management of pest populations of *S. noctilio* in the Southern Hemisphere is the parasitic nematode *Deladenus siricidicola* (Hajek and Morris, 2014; Slippers et al., 2015). *Deladenus siricidicola* was identified in New Zealand in 1962, putatively after an accidental introduction from Europe. Characterisation and screening of numerous strains resulted in the selection of a virulent strain from Hungary (the Sopron strain) for deployment in biological control programs in Australia (Bedding, 1972; Bedding and Akhurst, 1974; Bedding and Iede, 2005). The strain was highly effective, often sterilizing more than 90% of females in inoculated logs (Bedding and Akhurst, 1974). Continuous mass rearing in culture on *Amylostereum areolatum*, the fungal symbiont of *S. noctilio*, is thought to have eventually led to the loss of virulence in this strain (Bedding and Iede, 2005; Eskiviski et al., 2003, 2004). A strain was then obtained from sites where the Sopron strain was originally released, in the Kamona forest in Tasmania. The ‘Kamona strain’ has since been released in Australia, South America and South Africa in the 1990’s (Hurley et al., 2007). Apart from the Kamona strain, a strain referred to as ‘Encruzilhado do Sol’, isolated from infested wasps in Brazil, has been used in biological control programs in Brazil and Argentina (Eskiviski et al., 2003).

The diversity of *D. siricidicola* used in biological control programs stands in contrast to the diversity of invasive *S. noctilio* against which it is deployed. Mlonyeni et al. (2011) have shown that the samples from the biological control programs were virtually all homozygous and identical across the Southern Hemisphere (Argentina, Brazil and South Africa). Low genetic diversity may have resulted from genetic bottlenecks as well as inbreeding and selection during mass rearing and the distribution process. This low diversity in the nematode populations, and the potential lack of adaptability to different environments and different *S. noctilio* populations, might be one of the reasons that contribute to the variable success rate in inoculation programs using the nematode across the Southern Hemisphere (Hurley et al., 2008, 2007).

*Deladenus siricidicola* has been identified from invading populations of *S. noctilio* in Canada and the USA in the late 2000s (Kroll et al., 2013; Leal et al., 2012; Morris et al., 2013; Ryan et al., 2012; Yu et al., 2009). It appears that the nematode arrived with *S. noctilio* into both countries (Yu et al., 2009). Mitochondrial cytochrome oxidase subunit 1 (CO1) sequence data on *D. siricidicola* strains from North America showed that it is distinct from the Kamona strain (Leal et al., 2012). PCR-RFLPs also distinguished between the North American strains and the Southern Hemisphere strains (Mlonyeni et al., 2011; Morris et al., 2013). The North American strain is often referred to in literature as ‘non-sterilizing’, because the nematodes do not enter the eggs of the majority of wasps that they infect (Williams and Mastro, 2010; Yu et al., 2009). In contrast, the Kamona strain is nearly always found within eggs of *S. noctilio* wasps in the Southern Hemisphere (Bedding, 2009).

The impact of low genetic diversity in biocontrol populations of *D. siricidicola* on its efficacy in suppressing *S. noctilio* populations is not known. But it is plausible that the lack of additive variation might constrain local adaptation to variable climate and/or host tree or insect traits across its Southern Hemisphere range (Hufbauer and Roderick, 2005; Roderick and Navajas, 2003). Currently, very little is known regarding variation within or among populations of this nematode worldwide, despite its importance to global pine production (Slippers et al., 2012; Slippers et al., 2015). The aim of this study was to characterise global genetic diversity of *D. siricidicola* and to infer patterns of movement and spread, both intentional and accidental. Additionally,

**Table 1**Collection locations of the *Deladenus siricidicola* strains used in this study.

Country	Location	No. samples
Argentina	Misiones	1
Brazil	Santa Catarina	1
Canada	Ontario	9
Chile	Los Lagos	2
	BíoBío	1
	BíoBío	1
New Zealand	North Island, Rotoru-Kaingaroa Forest	2
South Africa	Eastern Cape	2
	Western Cape	2
	Southern Cape	3
	Kwazulu Natal	6
	Mpumalanga	8
Spain	Galicia	12
United States of America	Pennsylvania	6
	Vermont	1

this work contributes to efforts to catalog and store living, dormant strains of *D. siricidicola*, of which very few existed previously.

## 2. Material and methods

### 2.1. Nematode strains and DNA extraction

Fifty-seven nematode strains were collected from Argentina, Brazil, Canada, Chile, New Zealand, South Africa, Spain and United States of America (Table 1). They represent the presumed native range (Spain), areas of accidental introduction (Canada and the USA) and countries where *D. siricidicola* has been intentionally released to control *S. noctilio* (Argentina, Brazil, Chile, New Zealand and South Africa). Sample numbers collected from intentionally introduced regions were substantially higher due to easier access. In this study a strain represents a culture of nematodes isolated from a single wasp. All the strains used in this study are maintained at the Tree Protection Co-operative Program (TPCP) in the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

For DNA extraction, nematode cultures were reared in 500 ml Erlenmeyer flasks for one month. These flasks contained a mixture of sterilized wheat and rice and were inoculated with *A. areolatum* as a feeding substrate (Bedding and Akhurst, 1974). Nematodes were harvested from flasks using the method described by Mlonyeni et al. (2011). Briefly, the flasks were washed with sterile nanopure water and sieved. To allow nematodes to settle, the water was left for 30 min. The sediment was later pipetted into 1.5 ml Eppendorf tubes which were centrifuged at 13 000 rpm for 3 min. The supernatant was removed and the pellet used for DNA extractions.

DNA was extracted from the nematodes using a phenol–chloroform extraction method (Sambrook and Russell, 2006). Through phase separation proteins were eluted and DNA precipitated using 0.1 vol of 2 M NaAc and 2 volumes of 100% EtOH overnight. Samples were then centrifuged for 30 min at 8000 rpm (Eppendorf Centrifuge 5417C), followed by washing of the pellet with 70% EtOH. After vacuum drying (Concentrator 5301), pellets were resuspended in 50 µl Sabax water (Adcock Ingram Ltd., Bryamston, RSA). Digestion of RNA was done by adding 5 µl of RNaseA and incubating at room temperature (22 °C) for one hour. DNA concentrations of samples were determined using a ND-1000UV/Vis Spectrometer (Nanodrop Technologies, Wilmington, DE 19810 USA). Sample concentrations were adjusted to a final concentration of 100 ng/µl.

### 2.2. Confirmation of nematode identity

North American and Spanish collections are from areas where more than one *Deladenus* species co-occur. To confirm identity of the

collected strains, the mitochondrial cytochrome oxidase subunit 1 (CO1) gene region was sequenced for each strain. Primers CO1F and CO1R were used for PCR amplification (Morris et al., 2013). The PCR reaction mixture consisted of 5  $\mu$ l  $5 \times$  of MyTaq™ buffer, 0.5 M of each primer, 1.5 units of MyTaq™ DNA Polymerase (Biolone Ltd. UK), 2  $\mu$ l of the DNA template (approximately 200 ng) and sterile Sabax water to make up a final volume of 25  $\mu$ l. The PCR cycling procedure included 4 min initial denaturation at 95 °C followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension step of 72 °C for 45 min. From the PCR products 2  $\mu$ l were mixed with 1  $\mu$ l GelRed™ (Biotium, California) followed by electrophoresis on 2% (w/v) agarose gels in a TAE buffer system and visualized under ultraviolet light. PCR amplicons were purified using 6% Sephadex G-50 (Sigma-Aldrich, Germany) according to the manufacturer's instructions, followed by sequencing with the help of the Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA). Sequencing products were run on the ABI Prism™ 3500xl automated DNA sequencer (Applied Biosystems USA, Foster City, California). The sequences generated were submitted to GenBank (MF179729 – MF179784).

The available sequences of three described *Deladenus* spp. (*D. siricidicola*, *D. proximus* and *D. canii*) were obtained from GenBank and included in the dataset. Assembly of the sequencing reads was done in CLC Bio Main Workbench v6 (CLC Bio, [www.clcbio.com](http://www.clcbio.com)). MAFFT v7 was used to align the entire dataset, including sequencing data of described *Deladenus* spp. from GenBank, using the E-INS-I algorithm and additionally confirmed with ClustalW in Mega v6 (Tamura et al., 2011). To identify the best fit nucleotide substitution model, jModelTest v2.3.1 (Darriba et al., 2012; Guindon and Gascuel, 2003) was run. A maximum likelihood (ML) analysis was performed in PhyML v3.1 (Guindon and Gascuel, 2003). Branch support was calculated through 1000 bootstrap replicates. Additionally a maximum parsimony tree (MP) was constructed in PAUP v4.0b10 (Swofford, 2003) and tree length, consistency index (CI), rescaled consistency index (RC) and the retention index (RI) calculated. Furthermore to determine relatedness between populations in this study, a CO1 haplotype network based on the median-joining algorithm using NETWORK v4.6.1.3 (Bandelt et al., 1999) was constructed based on the 539 bp amplicon.

### 2.3. Microsatellite identification and primer design

Twelve microsatellite markers were used in this study. Ten were developed by Mlonyeni et al. (2011). The draft genome of *D. siricidicola* was used to identify two additional microsatellite repeats applying the program Msatcommander v0.8.2 (Faircloth, 2008) and flanking primers were designed using Primer 3.0 software (Rozen and Skaletsky, 1999). Ds33, a trinucleotide repeat (TGA), was amplified using primers Ds33F (5' CACCATCAACATCACCTCAT 3') and Ds33R (5' TGCTGTTGCGCAT TATCATT 3'). The second locus, Ds38, also a trinucleotide repeat (ACA) was amplified using the primers Ds38F (5' CGAGCGAAAACAACAAC AAC 3') and Ds38R (5' AGCATCATCATCCTCAGCATT 3'). All labelled primers for fragment analysis were sourced from Thermo Fisher Scientific (Life Technology, RSA). PCR reactions were set up as described for the CO1 gene region. The following PCR cycling parameters were applied: a 4 min. initial denaturation step at 95 °C followed by 35 cycles of denaturation at 95 °C at 30 sec., annealing at 58 °C for 30 sec., extension at 72 °C for 1 min. and a final extension of 72 °C for 45 min. followed by a cooling step to 4 °C for 10 min. The products were then visualized on 2% (w/v) agarose gels. Fluorescently labelled primers were arranged into two panels (Table 2) based on size and fluorescent dye colour, each consisting of either 7 or 5 primer pairs to conduct fragment analyses using GeneScan (Applied Biosystems, Foster City, California). PCR products were diluted to a ratio of 1:100 using Sabax water. The various amplicons from different primers were pooled according to the panel arrangement. 1  $\mu$ l of this pooled mix was added to 10  $\mu$ l of formamide and 0.2  $\mu$ l GeneScan-500 Liz size standard (Applied

Biosystems). These samples were run on the ABI PRISM™ 3500xl DNA analyser to determine product size (DNA sequencing facility, University of Pretoria). The software GeneMapper® v4.1 (Life Technologies, Foster City, CA) was run on the GeneScan data to score allele fragment sizes.

The different alleles were confirmed by sequencing the PCR fragments from strains that were homozygous for the alleles (Inqaba Biotech South Africa). This was done to confirm the amplification of the right locus and the variation in allele sizes observed. PCR and sequencing conditions were applied as described above. The sequences were submitted to GenBank (MF179717–MF179728).

### 2.4. Population structure analysis

Population structure amongst the *D. siricidicola* strains was inferred using Bayesian clustering principles in the program STRUCTURE v2.2 (Pritchard et al., 2000). A correlated allele frequency model allowing for admixture was performed. Burn-in length was set to 100 000 followed by a run length of 700 000 simulations. Twenty iterations were performed for each value of  $K$  (from  $K = 1$  to  $K = 10$ ), where  $K$  is the number of distinct populations. The optimal value for  $K$  was determined using the Evanno's method (Evanno et al., 2005) based on  $\Delta K$  and visualized using both STRUCTURE Harvester (Earl, 2012), as well as CLUMPAK (Kopelman et al., 2015). For each run of  $K$  CLUMPAK identifies possible major and minor modes representing alternative clustering of samples. We also performed a principal coordinate analysis (PCoA) using GENALEX v6.2 with the average genetic distance among population to further consider possible population structure (Peakall and Smouse, 2006).

### 2.5. Genetic diversity based on microsatellites

The presence of unique alleles, number of effective alleles, as well as the gene diversity (Nei, 1973) were investigated using GENALEX v6.2 (Peakall and Smouse, 2006). Genotypic diversity ( $\hat{G}$ ) indices were calculated by applying Stoddard and Taylor's method (1988) to the full dataset, while analyses of genetic diversity were performed on the clone-corrected dataset by removing identical genotypes. We compared genetic diversity among populations using sample rarefaction at  $N = 4$  to account for differences in strain number/sampling intensity. Observed and expected genetic diversity ( $H$ ),  $G'$  and Jost's  $D$  (Jost, 2008) were computed both within and among populations, using GENODIVE to determine differentiation among populations.

### 2.6. Scenario testing

Population introduction history was inferred using DIYABC (Cornuet et al., 2014, 2008). Using historical data on the timing and directionality of *D. siricidicola* introductions and population clustering results from STRUCTURE, the number of hypothesized introduction scenarios tested was limited to 11. For these analyses Chile, North America, Southern Hemisphere and Spain were defined as separate populations. An unsampled population as the source for all defined populations was also considered. In other scenarios, the source was represented by one of the defined populations from which the remaining populations diverged.

Default prior parameter settings were applied using the DIYABC program. The set of microsatellite markers used were either di- or trinucleotide repeats with different mutation rates. As such, two different mutation models were used according to the group of repeats. For dinucleotide repeats, the mean mutation rate was set at  $1E^5 - 1E^4$  and the individual locus mutation rate at  $1E^5 - 1E^4$ . For trinucleotide repeats, the mean mutation rate was set at  $1E^5 - 1E^4$  and the individual locus mutation rate at  $1E^5 - 1E^4$  selected based on a study on the nematode *Pristionchus pacificus* (Molnar et al., 2012). For the one sample summary statistics, the mean number of alleles, mean genetic diversity and mean size variance were selected. For two-sample summary statistics the  $F_{st}$ ,

**Table 2**  
Microsatellite primers used for the fragment analysis, their fluorescent labels and the panel arrangement.

SSR locus	SSR Motif	Forward primer 5'-3' Reverse primer 5'-3'	Panel No.	Fluorescent label	Tm (°C)
Ds1	GTA	CAATGTGCTGCGTCAATTT ACCCAACGCGTAGTGATAGC	1	FAM	59
Ds33	TGA	CACCATCAACATCACCTCAT TGCTGTGCGCATTATCATT	1	FAM	60
Ds38	ACA	CGAGCGAAAACAACAACAAC AGCATCATCATCCTCAGCATT	2	VIC	60
Ds54	CA	CAGCCACAACAATTACACC GCACAAAATCTCGCCTCAT	2	FAM	59
Ds105	AG	TGGTAGCAATCGATCGAAAA CGTGTCCACTTGCCCTCTC	1	NED	59
Ds201	TG	TGCATAGCTGGCGATAAATG CGAGTCACGTACGCATTAGC	2	NED	59
Ds316	GA	TGCGGATATCTTCTCATTGTAA TCAAATGTTATGGCAAATCTG	2	VIC	59
Ds323	TG	TTTACCTGTGGCTGTTACCG TGGGGTAAAAGTGGATTGGA	1	VIC	60
Ds325	GT	ACGCTTATGTGTGCCACTTG GGGTCTTGTGATGATGTTTCG	2	PET	59
Ds366	GT	CGCTGCTGTACTGCTGTTTT CACACAAATGCACACATGGA	1	PET	59
Ds375	AC	GGCAGCTGAAATGATGACAA ATCATCATCAGCAATATCCTCA	1	VIC	60
Ds388	(GT)(TG)	AAGTCAGCTGAAAAGCGGAAG TGTGTGCATGAAAACGGAAAC	1	NED	60

classification index, shared allele distance and  $(d)^2$  distance were chosen. To evaluate prior scenario combinations 1 000 000 simulated data sets were generated and used, with the help of principal component analysis (PCA). Posterior probabilities of the 11 scenarios were analysed using the closest 1% simulated data sets and selected summary statistics. Logistic regression analysis was applied to determine the confidence in scenario choice (Cornuet et al., 2014).

### 3. Results

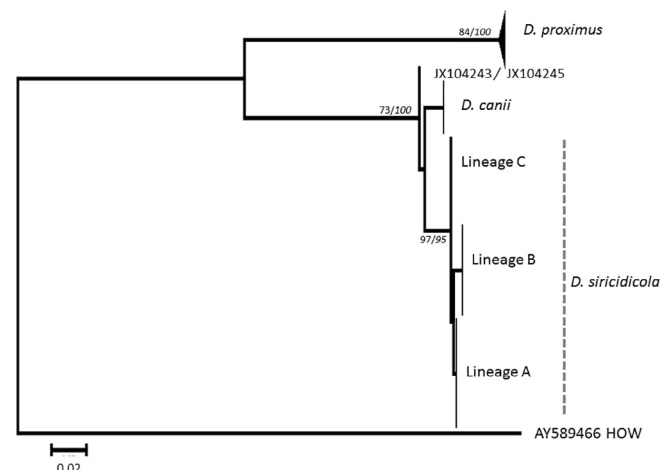
#### 3.1. Confirmation of nematode identity

The alignment of the cytochrome oxidase subunit 1 (CO1) fragment consisted of 539 base pairs including 59 parsimony-uninformative, 70 parsimony-informative and 410 constant characters. The 1000 most parsimonious trees were identified (TL = 0.93, CI = 0.87, RI = 0.99, and RC = 0.86). The phylogenetic analysis assigned all 57 nematode strains used in this study to *D. siricidicola* (Fig. 1).

The strains were separated into three haplotypes based on three informative sites (Fig. 2). Haplotype and nucleotide diversity was low (Hd = 0.343, Pi = 0.002). Each haplotype could be linked to distinct geographic regions. The first lineage (A) included all the strains from North America and Chile. The second lineage (B) included all but one of the strains from the Southern Hemisphere and the last lineage (C) represented all the strains from Spain, representing the native region. The solitary exception to this trend was that some Chilean strains grouped with the North American haplotype, while others grouped with the Southern Hemisphere haplotype.

#### 3.2. Microsatellite identification and primer design

In total 73 332 microsatellite regions were identified in the *D. siricidicola* genome. Of these microsatellites, the most abundant motif were the dinucleotides (31%) followed closely by the mononucleotide (30%). The trinucleotides made up 24%, the tetranucleotides 12% and the pentanucleotides and hexanucleotides combined constituted 3%. A total of 12 microsatellite markers were applied, including both dinucleotide and trinucleotide regions. Of the seven new microsatellite markers tested, two were polymorphic and were added to the existing



**Fig. 1.** Phylogenetic analysis of *Deladenus* spp. using both Maximum likelihood (ML) and Maximum Parsimony (MP) based on cytochrome c oxidase subunit 1 (CO1) sequence data. The tree is rooted to *Howardula aoronymphium*. All bootstrap values above 70% are indicated for ML and MP (italics) at the nodes.

ten microsatellite markers.

#### 3.3. Population structure analysis

STRUCTURE analysis grouped the data in two or three clusters (Fig. 3). According to Evanno's statistics (Evanno et al., 2005) the most likely number of populations ( $K$ ) was  $K = 2$ . However, taking the CO1 data and the historical background into consideration the most likely  $K$  was  $K = 3$ . The STRUCTURE analysis at  $K = 3$  differed from  $K = 2$  by splitting the Northern Hemisphere nematode strains into the North American lineage (Canada and United States) and the Spanish lineage. The Southern Hemisphere lineage consisted of strains from Argentina, Brazil, South Africa and New Zealand. The Chilean strains appeared to represent an admixed population of the North American and the Southern Hemisphere lineages. At  $K = 4$  the presence of a fourth lineage of unknown geographic origin was identified that seemed to be part of the admixed population in Chile. The Principal Coordinate

- South Africa
- Canada
- USA
- New Zealand
- Chile
- Spain
- Argentina
- Brazil

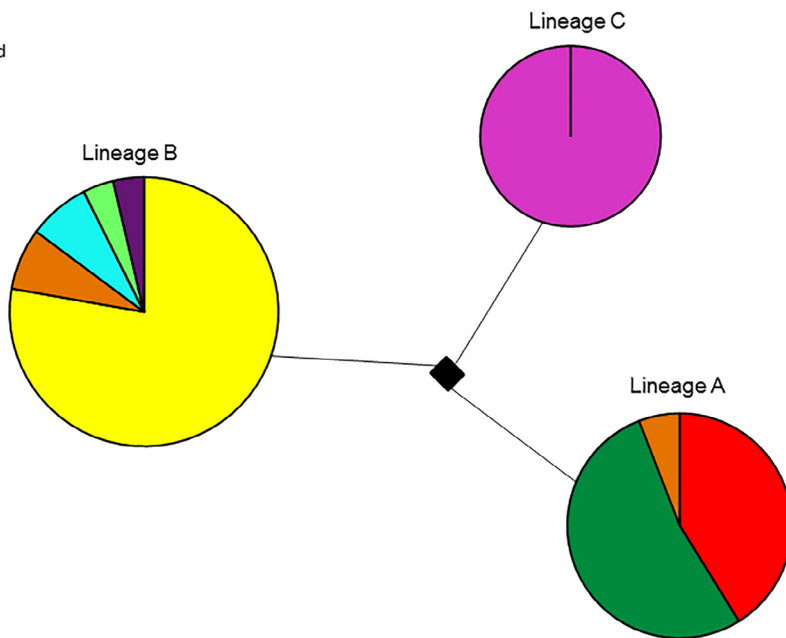


Fig. 2. A haplotype network based on analysis of the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene sequence data. The network was constructed using a median-joining algorithm. The circles represent the different haplotypes and the size of the circles is linked to the number of strains per haplotype. The colours represent the different geographic origins of the isolates.

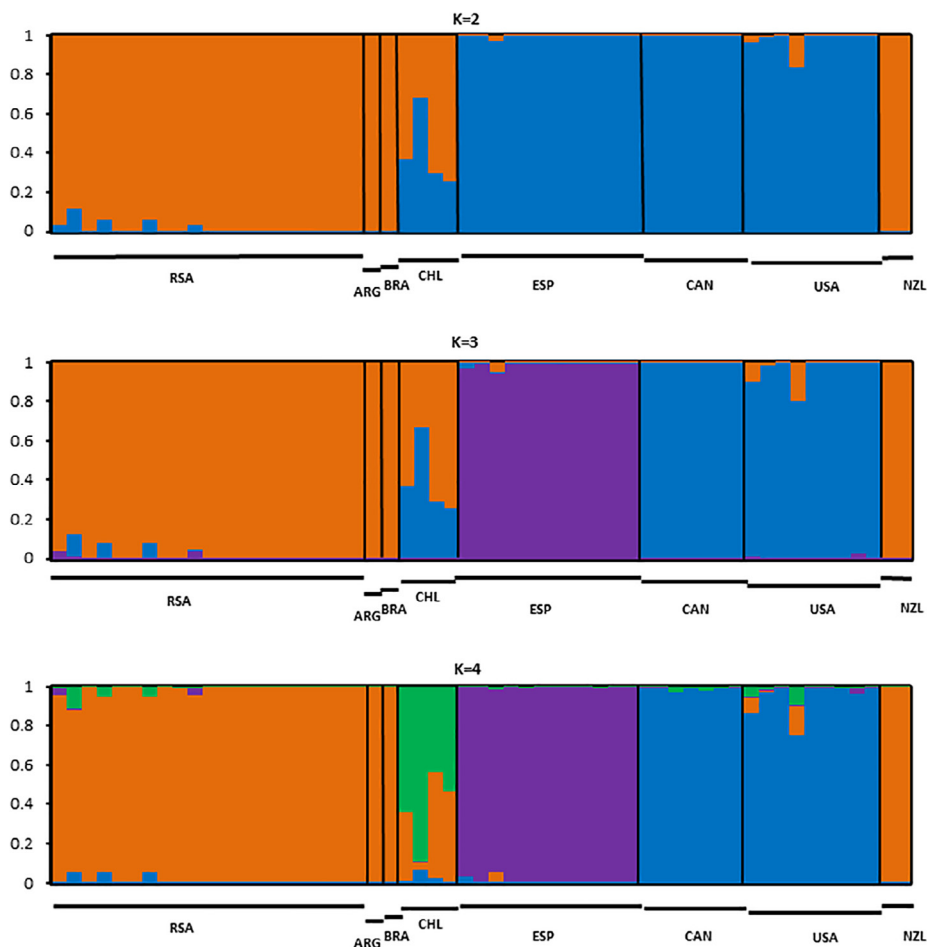
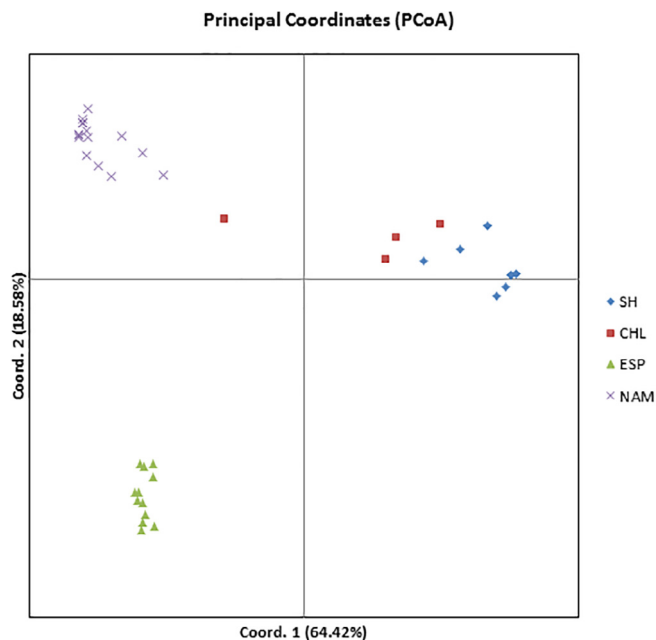


Fig. 3. Bayesian clustering analysis (STRUCTURE) conducted using microsatellite marker data from all isolates (n = 57). Vertical columns represent individual strains and the different colours represent identified populations. The countries of origin of the isolates are indicated by the horizontal lines. (ARG = Argentina, BRA = Brazil, CHL = Chile, CAN = Canada, ESP = Spain, NZL = New Zealand, RSA = South Africa and USA = United States of America).





**Fig. 4.** Principal coordinates analysis plot of the microsatellite data set using Nei's genetic distance (Nei, 1973). The different shapes represent the four different lineages (ESP = Spain, CHL = Chile, NAM = North America and SH = Southern Hemisphere). The first two principal coordinates provided the best separation.

Analysis separated the data into the same three clusters as STRUCTURE, with PC1 and 2 explaining 64.4% and 18.6% of the variation in the dataset respectively (Fig. 4).

### 3.4. Microsatellite diversity

Thirteen of the 134 alleles identified were unique to a specific lineage. The Spanish lineage had the highest number of unique alleles ( $n = 12$ ), whereas the Southern Hemisphere lineage had no unique alleles (Table 3). Gene diversity of the Southern Hemisphere lineage was the lowest ( $H = 0.119$ ), while the Chile lineage had the highest gene diversity ( $H = 0.500$ ) (Table 3). The same pattern was reflected in the genotypic diversity, whereby the Southern Hemisphere lineage showed the lowest diversity ( $e\hat{G} = 2.4$ ) and Chile had the highest rarefied genotypic diversity ( $e\hat{G} = 4$ , tied with Spain; Table 3).

Analysis of population differentiation using AMOVA showed that 86% of the molecular variation was due to variation among populations (Table 4). Pairwise comparison of the population diversity (Table 5) indicated the highest level of genetic differentiation between the North American and the Southern Hemisphere populations ( $G'' = 0.894$ ). The majority of alleles were shared between the Southern Hemisphere and Chilean populations indicating a lower level of genetic differentiation

**Table 3**  
Summary statistics of the populations in *D. siricidicola* using the microsatellite markers.

Population	No. of strains	No. of effective alleles	No. of unique alleles	No. of genotypes	Gene diversity <sup>a</sup>		Genotypic diversity	
					$H_o$	$H_e$	$\hat{G}^b$	$e\hat{G}^c$
Southern Hemisphere	25	1.08	0	7	0.119	0.109	3.2	2.4
Chile	4	1.76	2	4	0.500	0.458	4.0	4.0
Spain	12	1.42	11	12	0.167	0.210	12.0	4.0
North America	16	1.30	6	12	0.188	0.183	9.1	3.7

<sup>a</sup> The gene diversity calculated using all microsatellite markers based on Nei (1973).

<sup>b</sup> The method of Stoddart and Taylor (1988) was applied to estimate genotypic diversity ( $\hat{G}$ ).

<sup>c</sup>  $e\hat{G}$  refers to the genotypic diversity obtained using rarefaction based on the lineage with the smallest number of samples, in this study being Chile with four individuals.

( $G'' = 0.584$ ; Table 5).

The microsatellite data generated were used to construct a genotype network in which 38 genotypes were identified (Fig. 5). These genotypes grouped into three major clusters representing the North American population (Canada and United States of America), the Spanish population and the Southern Hemisphere population (Argentina, Brazil, New Zealand and South Africa). The Chilean strains grouped in between the Southern Hemisphere and the North American clusters.

### 3.5. Scenario testing

The best support in terms of posterior probability [ $P = 0.773$ ; 98%, (CI) 0.762–0.783], using Approximate Bayesian computation (DIYABC), strongly favoured a model in which 1) the Southern Hemisphere and the Spanish populations diverged first from an unsampled population, 2) where the Spanish population later gave rise to the North American population, and 3) where the Chilean population diversity arose via admixture between North American and Southern Hemisphere populations (Fig. 6).

## 4. Discussion

This is the first study to consider the genetic diversity of *D. siricidicola* at a global scale, including nematodes from the presumptive native range in Europe. Three lineages were identified that mapped to North America (Lineage A), the Southern Hemisphere (Lineage B, except for Chile which grouped with both Lineages A and B) and Spain (Lineage C). Interestingly, strains from Chile represented an admixed sample of both the Southern Hemisphere and North American lineages. The results re-affirm the lack of diversity in Southern Hemisphere populations of the nematode. They also provide a framework for understanding of the histories of introduction and invasion, and suggest opportunities to strategically amend biological control programs with additional genetic diversity.

All the datasets emerging from this study showed a clear distinction between the strains from North America and those from other areas of the world. The North American lineage (Lineage A) included strains from the USA and Canada and all twelve loci investigated could discriminate these strains from those collected in Spain and the Southern Hemisphere. North American samples were also represented by only one CO1 sequence haplotype, which differed from that of the Southern Hemisphere and the Spanish populations. *Deladenus siricidicola* was discovered soon after the first reports of *S. noctilio* in New York in 2005 (Hoebeke et al., 2005) when it was reported in 2006 from parasitized *S. noctilio* (Shields, 2009; Williams et al., 2009; Yu et al., 2009). Subsequent studies highlighted the distinction between North American strains and strains from the Southern Hemisphere (Leal et al., 2012; Mlonyeni et al., 2011; Morris et al., 2013). Since these populations so clearly differ genetically from Southern Hemisphere populations, it is prudent to avoid generalizations from studies on nematodes in North America when considering populations of *D. siricidicola* elsewhere in

**Table 4**  
Molecular variance analysis (AMOVA) of microsatellite marker data from all *Deladenus siricidicola* strains and populations.

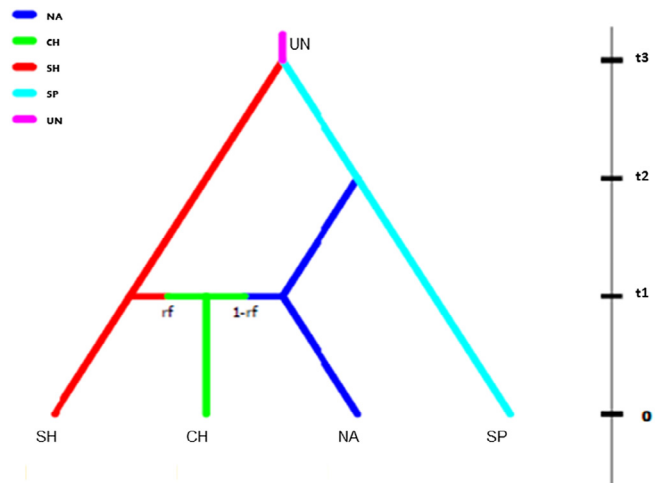
Source	df	Estimated variance	Proportion of variance (%)	Statistic	Value	Probability
Among populations	3	590.3	86%	$\Phi_{PT}$	0.864	0.001
Within populations	53	125.7	14%			
Total	56	716.0	100%			

**Table 5**  
Pairwise genetic difference values amongst the four populations. The upper right corner shows the Jost's D values and the lower left values are G''.

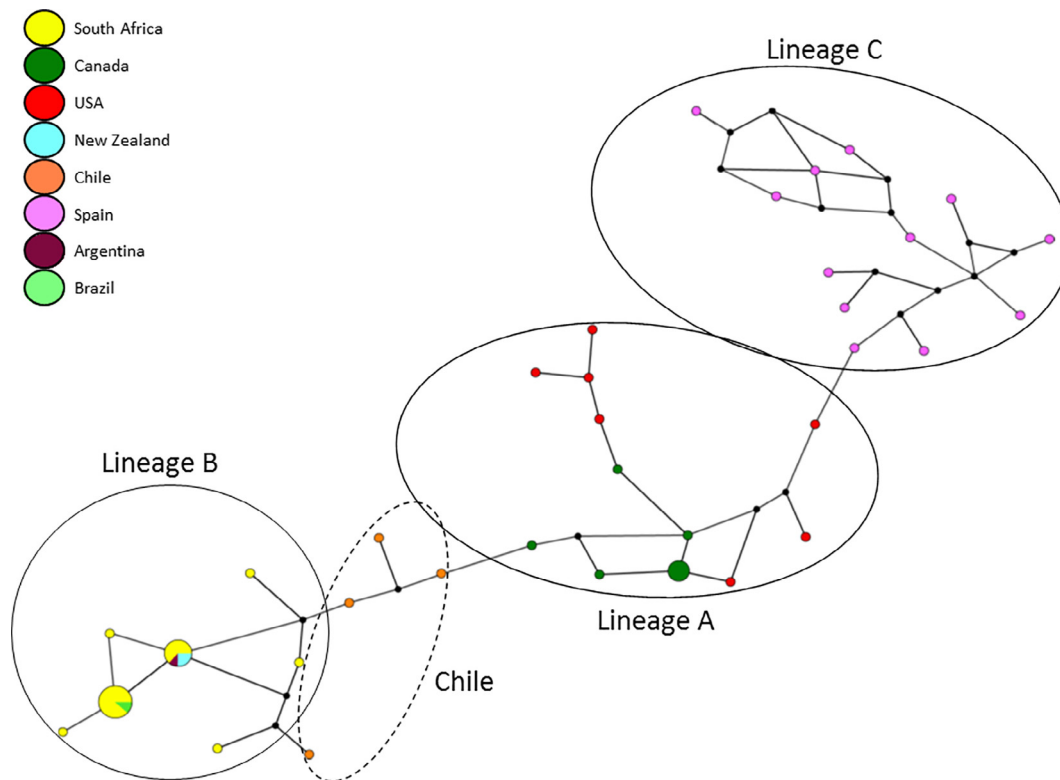
	Southern Hemisphere	Chile	Spain	North America
Southern Hemisphere	–	0.188	0.898	0.965
Chile	0.584	–	0.727	0.560
Spain	0.882	0.648	–	0.498
North America	0.894	0.636	0.684	–

the world, and *vice versa*. Biocontrol programs using *D. siricidicola* should ideally be studied in the context and environment where they are intended to be used and in relation to local fungal and Siricid populations as well as local climatic conditions.

The lack of diversity in Southern Hemisphere populations is not surprising since these biocontrol strains were isolated from a single source (Tasmania, Australia, where biocontrol strains collected from Hungarian wasps were originally released) and shared with countries and pine growers. However, the relatively high genetic diversity in the North American population was unexpected. Diversity in North America was comparable to that seen in the Spanish population, which represents part of the presumed native region. There have been no reports of intentional introductions of *D. siricidicola* strains in North America; the species is assumed to have arrived with the *S. noctilio*



**Fig. 6.** Representation of the most likely scenario ( $P = 0.79$ ) among the 11 scenarios tested in our DIYABC analysis. The figure represents the most likely scenario (SP = Spain, CH = Chile, NA = North America, SH = Southern Hemisphere and UN = Unsampled region). The vertical axis represents time but is not to scale.



**Fig. 5.** Genotype network drawn using microsatellite markers. The network is based on a median-joining algorithm. Coloured circles represent the different genotypes and the size of the circles is linked to number of strains with that genotype. The colours represent the different geographic origins of the isolates. The small black circles indicate undetected intermediate genotypes. Solid lines are used to group the genotypes into the three lineages, while the dashed line groups the Chilean strains.

introduction into North America (Bittner et al., 2017; Yu et al., 2009). From this perspective, such high diversity is intriguing, especially given the likelihood of a bottleneck (Roderick and Navajas, 2003). Work done on the fungal symbiont *A. areolatum* (Bergeron et al., 2011; Castrillo et al., 2015; Nielsen et al., 2009) and *S. noctilio* (Boissin et al. 2012; Bittner et al. 2017) suggests multiple introductions of the wasp into North America. This suggests that there could have also been multiple introductions of *D. siricidicola* into North America. In the case of the wasp and the fungus these introductions appear to have come from at least two distinct geographic areas, whereas the North American strains of *D. siricidicola* appear to come from a single geographic origin. Such unexpectedly high levels of diversity, possibly due to multiple or large introductions, appears to be a pattern amongst many invasive pests (Garnas et al. 2016). A broader *D. siricidicola* population study across North America would allow for a better understanding of origin and number of introductions of the nematode in that region of the world.

The origin of the North American population of *D. siricidicola* cannot be conclusively inferred from the results of this study. However, DIYABC analyses suggests that the North American lineage is derived from the Spanish lineage (Lineage C) and is related but not identical to our Spanish samples. Previous studies on the introduction of *S. noctilio* and *A. areolatum* into North America support an introduction from Europe (Bergeron et al., 2011; Bittner et al., 2017; Boissin et al., 2012; Nielsen et al., 2009) though a second introduction from an unknown source, possibly South America, is likewise supported (Boissin et al. 2012).

The existence of a single lineage (B) of *D. siricidicola* in Brazil, Argentina, New Zealand and South Africa was expected given the extensively deployed biological control program across these regions from a single source population. The Kamona strain of the nematode has been repeatedly released in these countries since the early 2000's (Bedding and Iede, 2005; Hurley et al., 2007; Slippers et al., 2012). The effect of this reduced diversity present in the Southern Hemisphere should be considered in light of (1) the diversity of the *S. noctilio* populations across the Southern Hemisphere (Boissin et al. 2012), (2) variation in interactions between nematode strains and *S. noctilio* populations (Bedding and Iede, 2005), and (3) the diversity of tree species and environmental conditions across this range (Hurley et al., 2007). Furthermore, the small sample sizes are unlikely to represent the full regional diversity and further sampling in these regions would add valuable information.

The Southern Hemisphere lineage (B) had the lowest genetic diversity of all the regions in this study and contained no private alleles. Low diversity (and high homozygosity, also observed in this study and in Mlonyeni et al., 2011) suggests some degree of inbreeding. This is likely to have occurred given the serial sub-culturing and intensive rearing inherent to this and other augmentative biological control programs (Bedding and Iede, 2005; Slippers et al., 2012).

Isolates from Chile represented an admixed population containing diversity from both the North American and Southern Hemisphere lineages. While this population had a low number of unique alleles, it had the highest level of gene diversity of all populations sampled, likely as a direct consequence of admixture. Historically, Chile obtained *D. siricidicola* strains from Brazil and New Zealand for use in their biological control program, with no known intentional introduction from North America (personal observation). Thus, it appears that there might have been an accidental introduction of *D. siricidicola* from North America into Chile. An alternative hypothesis is that other source populations of the Chilean population share diversity with North American populations. This finding aligns with the potential link in populations of *S. noctilio* in North America and South America (Boissin et al., 2012, Bittner et al., 2017).

The population of *D. siricidicola* from Spain was more diverse than other populations in this study. It also appears to be part of the population of origin of the North American populations of the nematode. Spain forms part of the presumed native range of *D. siricidicola*, which

extends across Europe and also includes parts of Asia (Bedding and Akhurst, 1978). While the nematode is thought to have been introduced into North America (Bittner et al., 2017; Yu et al., 2009), the high diversity in that country could suggest a native origin, or at least a longer biogeographic history. Because our sampling in the native range of *S. noctilio* was limited, fine scale phylogeographic interpretations were not possible. Broader studies of *D. siricidicola* diversity across Eurasia would be needed to understand the origin of introduced populations. Furthermore, Lombardero et al. (2016) shows that the Spanish population of *D. siricidicola* sterilizes the local *S. noctilio* female wasps, whereas the North American strains of *D. siricidicola* do not sterilize the local *S. noctilio* (Williams and Mastro, 2010; Yu et al., 2009). It would be interesting to consider in future studies to what extent this variation in the sterilization ability of the nematode is genetically determined and conserved versus being affected by environment or subject to rapid evolutionary change.

## 5. Conclusions

The use of molecular markers has made it possible to identify three distinct lineages amongst *D. siricidicola* global populations. Consequently, it has been possible to characterise and confirm patterns of spread around the world. The markers developed in this study and the curated strains that now reside in long-term storage provide tools and raw material for further exploration of the diversity of this important biological control agent of *S. noctilio*. Such studies hold the ultimate goal of developing increasingly effective and resilient biological control programs. The discovery of admixture in the Chile population of *D. siricidicola* confirms that inter-lineage cross-breeding is possible and occurs with some frequency in the wild. This has relevance both for efforts to increase diversity in biological control programs, for example by selecting or breeding high-efficiency strains for specific regions and pest populations. The effects of the genetic diversity of *D. siricidicola* on the effectiveness of biological control programs remains to be investigated, and can now be directed using the information provided in this study.

## CRedit authorship contribution statement

**Katrin N.E. Fitza:** Investigation, Data curation, Writing - original draft. **Jeff R. Garnas:** Supervision, Project administration, Funding acquisition. **Maria J. Lombardero:** Resources. **Matthew P. Ayres:** Resources. **Flora E. Krivak-Tetley:** Resources. **Rodrigo Ahumada:** Resources. **Brett P. Hurley:** Supervision, Project administration, Funding acquisition. **Michael J. Wingfield:** Supervision, Project administration, Funding acquisition. **Bernard Slippers:** Supervision, Project administration, Funding acquisition.

## Acknowledgements

The study was made possible through funding from the University of Pretoria, members of the Tree Protection Co-operative Program (TPCP), South Africa, and USDA Forest Service International Programs. We acknowledge the support by the Sequencing Facility of the University of Pretoria for their services related to the fragment analyses.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2019.01.014>.

## References

- Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48.
- Bedding, R., 1972. *Biology of Deladenus siricidicola* (Neotylenchidae) an entomophagous-



- mycetophagous nematode parasitic in siricid woodwasps. *Nematologica* 18, 482–493.
- Bedding, R., Akhurst, R., 1974. Use of the nematode *Deladenus siricidicola* in the biological control of *Sirex noctilio* in Australia. *Aust. J. Entomol.* 13, 129–135.
- Bedding, R., Akhurst, R., 1978. Geographical distribution and host preferences of *Deladenus* species (Nematoda: Neotylenchidae) parasitic in siricid woodwasps and associated hymenopterous parasitoids. *Nematologica* 24, 286–294.
- Bedding, R., Iede, E., 2005. Application of *Beddingia siricidicola* for *Sirex* woodwasp control. *Nemat. Biocontrol Agent.* 385–399.
- Bedding, R. A., 2009. Controlling the pine-killing woodwasp, *Sirex noctilio*, with nematodes. In: *Use of Microbes for Control and Eradication of Invasive Arthropods* pp. 213–235.
- Bergeron, M.J., Leal, I., Foord, B., Ross, G., Davis, C., Slippers, B., de Groot, P., Hamelin, R.C., 2011. Putative origin of clonal lineages of *Amylostereum areolatum*, the fungal symbiont associated with *Sirex noctilio*, retrieved from *Pinus sylvestris*, in eastern Canada. *Fungal Biol.* 115, 750–758.
- Bittner, T.D., Hajek, A.E., Haavik, L., Allison, J., Nahrung, H., 2017. Multiple introductions of *Sirex noctilio* (Hymenoptera: Siricidae) in northeastern North America based on microsatellite genotypes, and implications for biological control. *Biol. Invasions* 19, 1431–1447.
- Boissin, E., Hurley, B., Wingfield, M., Vasaitis, R., Stenlid, J., Davis, C., Groot, P.d., Ahumada, R., Carnegie, A., Golderazena, A., 2012. Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp. *Mol. Ecol.* 21, 5728–5744.
- Booy, G., Hendriks, R., Smulders, M., Groenendaal, J.v., Vosman, B., 2000. Genetic diversity and the survival of populations. *Plant Biol.* 2, 379–395.
- Castrillo, L.A., Hajek, A.E., Pajares, J.A., Thomsen, L.M., Csóka, G., Kenaley, S.C., Kepler, R.M., Zamora, P., Angeli, S., 2015. Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US. *Fungal Biol.* 119, 595–604.
- Cornuet, J.-M., Pudlo, P., Veysier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.-M., Estoup, A., 2014. DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics* 30, 1187–1189.
- Cornuet, J.-M., Santos, F., Beaumont, M.A., Robert, C.P., Marin, J.-M., Balding, D.J., Guillemaud, T., Estoup, A., 2008. Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. *Bioinformatics* 24, 2713–2719.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9, 772.
- Earl, D.A., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4, 359–361.
- Eskiviski, E., De Coll, O. d. R., Faraldo, G., Bennesch, M., 2003. Efficiency of new isolations of *Deladenus siricidicola* B. in the biological control of *Sirex noctilio*. In “11as Jornadas Técnicas Forestales y Ambientales”, EEA Monetecarlo, INTA.
- Eskiviski, E. R., Faraldo, G., de Coll, O. d. R. 2004. Effects of the nematode *Beddingia* (= *Deladenus*) *siricidicola* parasitism in adults stage of wood wasp *Sirex noctilio*. In “11as Jornadas Técnicas Forestales y Ambientales”, EEA Monetecarlo, INTA.
- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14, 2611–2620.
- Faircloth, B.C., 2008. Msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol. Ecol. Resour.* 8, 92–94.
- Forsman, A., 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. *Proc. Natl. Acad. Sci.* 111, 302–307.
- Garnas, J.R., Auger-Rozenberg, M.-A., Roques, A., Bertelsmeier, C., Wingfield, M.J., Saccaggi, D.L., Roy, H.E., Slippers, B., 2016. Complex patterns of global spread in invasive insects: eco-evolutionary and management consequences. *Biol. Invasions* 18, 935–952.
- Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52, 696–704.
- Hajek, A., Morris, E., 2014. Biological control of *Sirex noctilio*. In: R.G. Van Driesche R. Reardon, (Eds.) *The Use of Classical Biological Control to Preserve Forests in North America*. FHTE-2013-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia, PP. 331–346.
- Hoebeke, E.R., Haugen, D.A., Haack, R.A., 2005. *Sirex noctilio*: discovery of a paleartic siricid woodwasp in New York. *Newslett. Michigan Entomol. Soc.* 50, 24–25.
- Holt, R.D., Hochberg, M.E., 1997. When is biological control evolutionarily stable (or is it)? *Ecology* 78, 1673–1683.
- Hufbauer, R.A., 2002. Evidence for nonadaptive evolution in parasitoid virulence following a biological control introduction. *Ecol. Appl.* 12, 66–78.
- Hufbauer, R.A., Roderick, G.K., 2005. Microevolution in biological control: mechanisms, patterns, and processes. *Biol. Control* 35, 227–239.
- Hurley, B.P., Slippers, B., Croft, P.K., Hatting, H.J., van der Linde, M., Morris, A.R., Dyer, C., Wingfield, M.J., 2008. Factors influencing parasitism of *Sirex noctilio* (Hymenoptera: Siricidae) by the nematode *Deladenus siricidicola* (Nematoda: Neotylenchidae) in summer rainfall areas of South Africa. *Biol. Control* 45, 450–459.
- Hurley, B.P., Slippers, B., Wingfield, M.J., 2007. A comparison of control results for the alien invasive woodwasp, *Sirex noctilio*, in the Southern Hemisphere. *Agric. For. Entomol.* 9, 159–171.
- Joron, M., Brakefield, P.M., 2003. Captivity masks inbreeding effects on male mating success in butterflies. *Nature* 424, 191–194.
- Jost, L., 2008. GST and its relatives do not measure differentiation. *Mol. Ecol.* 17, 4015–4026.
- Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* 15.
- Kroll, S.A., Hajek, A.E., Erin Morris, E., Long, S.J., 2013. Parasitism of *Sirex noctilio* by non-sterilizing *Deladenus siricidicola* in northeastern North America. *Biol. Control* 67, 203–211.
- Leal, I., Foord, B., Davis, C., de Groot, P., Mlonyeni, X., Slippers, B., 2012. Distinguishing isolates of *Deladenus siricidicola*, a biological control agent of *Sirex noctilio*, from North America and the Southern Hemisphere using PCR-RFLP. *Can. J. For. Res.* 42, 1173–1177.
- Lombardero, M., Ayres, M., Krivak-Tetley, F., Fitz, K., 2016. Population biology of the European woodwasp, *Sirex noctilio*, in Galicia, Spain. *Bull. Entomol. Res.* 106, 569–580.
- Mlonyeni, X.O., Wingfield, B.D., Wingfield, M.J., Ahumada, R., Klasmer, P., Leal, I., De Groot, P., Slippers, B., 2011. Extreme homozygosity in Southern Hemisphere populations of *Deladenus siricidicola*, a biological control agent of *Sirex noctilio*. *Biol. Control* 59, 348–353.
- Molnar, R.I., Witte, H., Dinkelacker, I., Villate, L., Sommer, R.J., 2012. Tandem-repeat patterns and mutation rates in microsatellites of the nematode model organism *Pristionchus pacificus*. *G3: Genes Genomes*. *Genetics* 2, 1027–1034.
- Morris, E.E., Kepler, R.M., Long, S.J., Williams, D.W., Hajek, A.E., 2013. Phylogenetic analysis of *Deladenus* nematodes parasitizing northeastern North American *Sirex* species. *J. Invertebr. Pathol.* 113, 177–183.
- Nei, M., 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* 70, 3321–3323.
- Nielsen, C., Williams, D.W., Hajek, A.E., 2009. Putative source of the invasive *Sirex noctilio* fungal symbiont, *Amylostereum areolatum*, in the eastern United States and its association with native siricid woodwasps. *Mycol. Res.* 113, 1242–1253.
- Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* 6, 288–295.
- Phillips, C., Baird, D., Iline, I., McNeill, M., Proffitt, J., Goldson, S., Kean, J., 2008. East meets west: adaptive evolution of an insect introduced for biological control. *J. Appl. Ecol.* 45, 948–956.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- Reed, D.H., Frankham, R., 2003. Correlation between fitness and genetic diversity. *Conserv. Biol.* 17, 230–237.
- Roderick, G.K., Navajas, M., 2003. Genes in new environments: genetics and evolution in biological control. *Nat. Rev. Genet.* 4, 889–899.
- Rozen, S., Skaletsky, H., 1999. Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols*. Springer, pp. 365–386.
- Ryan, K., De Groot, P., Nott, R., Drabble, S., Ochoa, I., Davis, C., Smith, S., Turgeon, J., 2012. Natural enemies associated with *Sirex noctilio* (Hymenoptera: Siricidae) and *S. nigricornis* in Ontario, Canada. *Environ. Entomol.* 41, 289–297.
- Sambrook, J., Russell, D.W., 2006. Purification of Nucleic Acids by Extraction with Phenol: Chloroform. *Cold Spring Harbor Protocols* prot4455.
- Shields, L., 2009. Proceedings. 19th US Department of Agriculture interagency research forum on invasive species. *Gen Tech Rep NRS-P-36*. USDA Forest Service, Newtown Square.
- Slippers, B., Hurley, B.P., Mlonyeni, X.O., Groot, P., Wingfield, M.J., 2012. Factors affecting the efficacy of *Deladenus siricidicola* in biological control systems. In: *The Sirex Woodwasp and its Fungal Symbiont: Research and Management of a Worldwide Invasive Pest*. Springer Science-Business Media, pp. 119–133.
- Slippers, B., Hurley, B.P., Wingfield, M.J., 2015. *Sirex* woodwasp: a model for evolving management paradigms of invasive forest pests. *Annu. Rev. Entomol.* 60, 601–619.
- Stoddart, J.A., Taylor, J.F., 1988. Genotypic diversity: estimation and prediction in samples. *Genetics* 118, 705–711.
- Stouthamer, R., Kazmer, D.J., 1994. Cytogenetics of microbe-associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. *Heredity* 73, 317–327.
- Swofford, D. 2003. PAUP\*: phylogenetic analysis using parsimony, version 4.0 b10.
- Szűcs, M., Melbourne, B.A., Tuff, T., Weiss-Lehman, C., Hufbauer, R.A., 2017. Genetic and demographic founder effects have long-term fitness consequences for colonising populations. *Ecol. Lett.* 20, 436–444.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Tomasetto, F., Tylianakis, J.M., Reale, M., Wratten, S., Goldson, S.L., 2017. Intensified agriculture favors evolved resistance to biological control. *Proc. Natl. Acad. Sci.* 114, 3885–3890.
- Williams, D.W., Mastro, V.C., 2010. 21st US Department of Agriculture Interagency Research Forum on Invasive Species 2010.
- Williams, D.W., Zylstra, K.E., Mastro, V. 2009. In: Proceedings. 20th US Department of Agriculture Interagency Research Forum on Invasive Species.
- Yu, Q., Groot, P.d., Leal, I., Davis, C., Ye, W., Foord, B., 2009. Characterization of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) associated with *Sirex noctilio* (Hymenoptera: Siricidae) in Canada. *Int. J. Nematol.* 19, 23–32. Further reading
- Manzana-Román, R., Oleaga, A., Pérez-Sánchez, R., Siles-Lucas, M., 2012. Gene silencing in parasites: current status and future prospects. *Adv. Parasitol.* 78, 1–55.