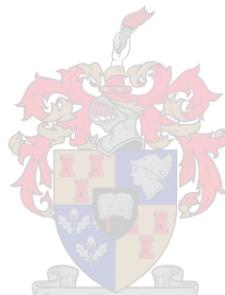


# Population genetics of the invasive wasp, *Vespula germanica*, in South Africa

by

**Julia Eloff**



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Department of Conservation Ecology and Entomology, Faculty of AgriSciences

*Supervisor:* Dr Ruan Veldtman  
*Co-supervisor:* Prof. Phil Lester

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## **Declaration**

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## Summary

The German wasp (*Vespula germanica*) is a highly successful invader on a global scale. These wasps were first observed in the Western Cape region in South Africa in 1972 and they have the potential to expand their range and cause significant damage to the native biodiversity. Our study used nuclear (microsatellites) and mitochondrial DNA (mtDNA) from 42 wasp colonies to analyse the population genetics of *V. germanica* in their invaded South Africa range. We sequenced three mitochondrial genes; cytochrome c oxidase I, cytochrome b and cytochrome c oxidase II. We found six mtDNA haplotypes present in South Africa, suggesting either multiple introductions or one introduction with multiple queens. We examined nine microsatellite loci and found weak to no genetic sub-structuring, likely due to high dispersal rates. We concluded that German wasps in South Africa maintain a homogenous population, most likely via movement of individuals between localities. Due to the presence of multiple introductions in the region, it could mean that future introductions may be likely and therefore eradication would be ineffective as recolonization would occur. It is therefore recommended that further efforts be made to prevent additional introductions prior to eradication efforts.

## Dedication

*To my dearest mom, Susan Ann Eloff (1952-2015), and dad, Sarel Johan Eloff (1949-2015).*

*Thank you for raising me to be resilient even through the hardest of times.*

*Without you I wouldn't be who and where I am today. I just wish you were here to see it and celebrate with me.*

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## **Preface**

This thesis is presented as a compilation of four chapters. Each chapter is written according to the style of the journal *Insectes Sociaux* to which a condensed version of this thesis was submitted and accepted for publication. The mitochondrial and microsatellite analyses, as submitted to the journal, were combined as they work concurrently to substantiate on findings.

### **Chapter 1                    General Introduction and Literature Review**

An overview of biological invasions of social insects, with focus on *Vespula germanica*, and the role of population genetics in invasion studies.

### **Chapter 2                    Materials and Methods**

Molecular analysis methods of the mitochondrial and nuclear DNA of *Vespula germanica* in South Africa.

### **Chapter 3                    Results**

Population genetics of the invasive wasp, *Vespula germanica* in South Africa.

### **Chapter 4                    General Discussion and Conclusions**

An overview of the population genetic structure of *Vespula germanica* in South African and future directions.

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# 1. Introduction and Literature Review

## 1.1. Biological invasions

An invasive species can be defined as a non-native species that has been introduced into a region in which, its introduction has negatively impacted the environment (Colautti and MacIsaac 2004). The successful establishment of invasive species can result in significant damage to their introduced ranges; including damage to economies, native biodiversity and human health (Pimentel et al. 2005; Kenis et al. 2009; Pyšek and Richardson 2010). With an increasing human population, and ongoing trade and mobility, anthropogenic changes to the environment have become more prevalent worldwide (Villemant et al. 2011). The accidental or intentional human-induced movement of non-native species is often attributed to causing the introduction of an invasive species (Hulme et al. 2008). The number of biological invasions that have occurred has drastically increased since the 18<sup>th</sup> century (Grosholz 2005). This increasing occurrence of invasions has led to increased efforts to eradicate and control these invasive species (Allendorf and Lundquist 2003). One of the most notorious examples of invasive success is the case of the yellow crazy ant (*Anoplolepis gracilipes* (Smith, 1857)) on Christmas Island (Wetterer 2005). The ant was accidentally introduced in the early 1900's and since the late 1990's has resulted in the death of millions of land crabs which are vital in maintaining the island's biodiversity (Wetterer 2005). They prey on the native species in the area and additionally cause tree dieback and affect forest composition and soil health (O'Dowd et al. 2014).

## 1.2. Social insects as invaders

Social insects, predominantly Hymenoptera, are classed globally as one of the most infamous invasive groups (Moller 1996; Chapman and Bourke 2001). Several social insect species are listed in the IUCN's list of the most invasive species worldwide (Lowe et al. 2004). Factors said to enhance invasive success of these social insects include their high reproductive rate and adept dispersal abilities, both through self- and human-mediated dispersal (Passera 1994; Moller 1996; Kenis et al. 2009). The small size of insects allows them to easily sneak into crevices and small spaces unnoticed in containers, airline cargo and other forms of transportation baggage. Another key characteristic in the successful establishment of social insects is their eusocial nature which has been linked to their

ecological dominance (Wilson and Hölldobler 2005). Social insects have the ability to form a large colony from a single inseminated queen (Chapman and Bourke 2001). The queens are able to store sperm from multiple matings in their spermathecae for a prolonged period, which can facilitate their successful establishment in a new location (Crozier and Page 1985; Keller and Reeve 1994; Moller 1996). When establishing new populations, the wasp's sex determination system drives the colony fate. Diploid individuals heterozygous at a single locus, complementary sex determiner (*csd*), develop as females whilst haploid individuals develop as males. Diploid individuals homozygotes at the *csd* locus develop as diploid males, but these do not survive to adulthood or cannot sire offspring (Beye et al. 2003). The costly production of diploid males is the result of inbreeding depression caused by reduced genetic variation post-bottleneck. Thus, introductions by a single monandrous queen that has mated with a male with a matching allele in the *csd* locus will not succeed (Ding et al. 2017). Polyandry can be beneficial and facilitate the successful establishment in a new location (Crozier and Page 1985; Keller and Reeve 1994; Moller 1996; Goodisman et al. 2002). The Australian invasion of the Asian honey bee, *Apis cerana* (Fabricius, 1793), occurred from a single introductory event in Cairns (Gloag et al. 2017). Balancing selection maintained heterozygosity at the *csd* locus by retaining rare *csd* alleles following the invasion, rescuing the population from a founder effect (Gloag et al. 2017). Another example is that of the bumble bee, *Bombus terrestris* (Linnaeus, 1758), that appears to have invaded Tasmania from a single inseminated queen, based on male diploidy prevalence (Buttermore et al. 1998). This polyandrous ability of queens to store sperm from multiple matings aids in invasion processes through the reduction of population variance in colony fitness as well as increasing heterozygosity at the sex locus (Gloag et al. 2017).

### **1.3. The German wasp; *Vespula germanica***

#### **1.3.1. Description and identification**

*Vespula germanica* (Fabricius, 1793), also known as the German wasp or European wasp, is native to Europe, North Africa and temperate Asia (Spradbery and Maywald 1992). This wasp is commonly confused for its sister species in its native range, *Vespula vulgaris* (Linnaeus, 1758), as they both have distinct black and yellow colourations. Unlike the common wasp (*Vespula vulgaris*), the German wasp can be identified by the presence of dots on the clypeus above the mandibles, and a complete yellow strip behind the eye

(Clapperton et al. 1989) (Figure 1). The wasp is on average 1.3 cm long with the abdomen divided into 6 or 7 segments (Kovac and Stabentheiner 2012). A wasp nest is generally comprised of a queen, workers and drones (males). Drone abdomens are longer than that of workers, whilst the head and thorax are similar in size. The queens have an abdomen that is broader but similar in length to that of a drone (Thomas 1960).

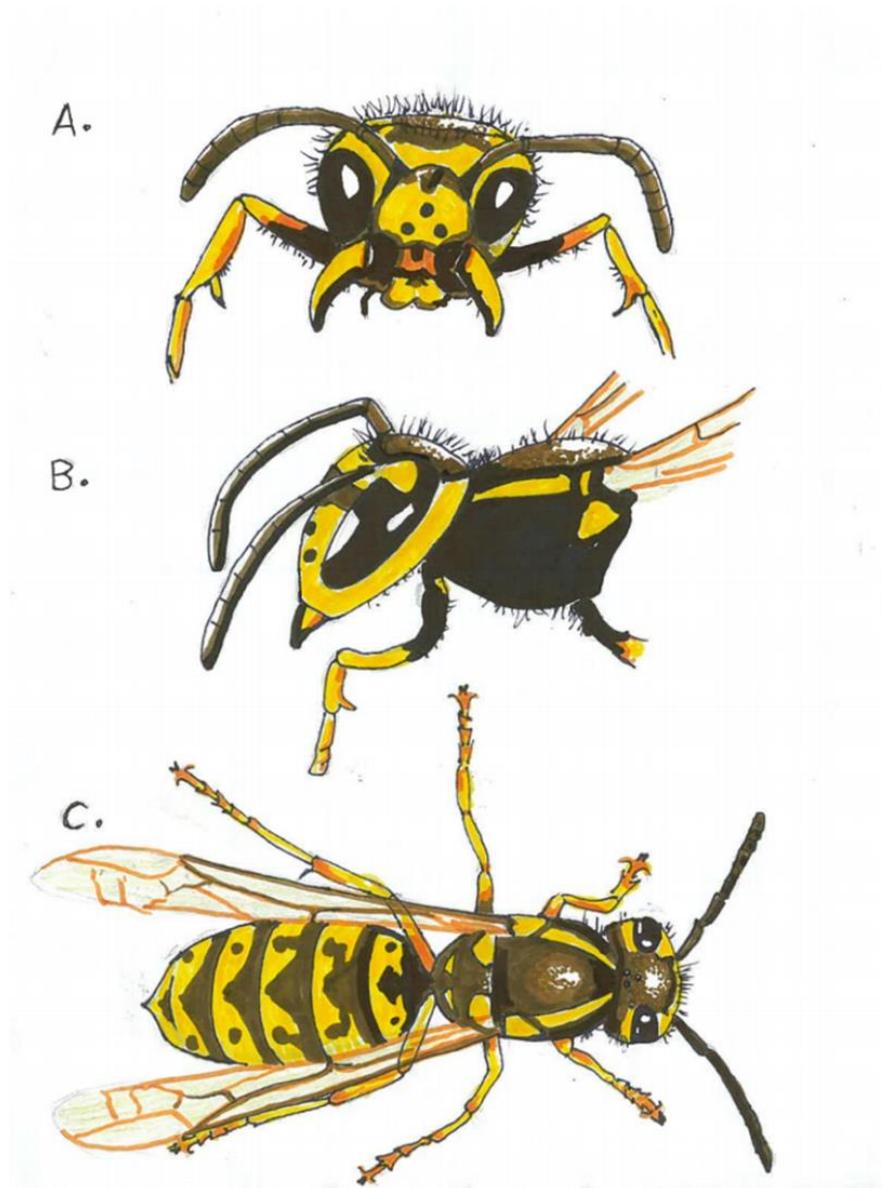


Figure 1. Illustration of *Vespula germanica* key characteristics which are represented by the presence of dots on the face (A), a clear yellow strip behind the eye (B). (C) Represents a full body top view of *V. germanica*. Illustrations by J. Eloff

### 1.3.2. Life history

German wasps are a eusocial species with an annual life cycle (Whitehead and Prins 1975; Kasper et al. 2008). Due to this eusocial nature, these wasps in a nest function as a unit, with tasks divided among one another- usually a reproductive caste and worker caste (Moller 1996). Worker tasks usually include, but are not limited to carbohydrate foraging, protein foraging, pulp foraging and nest work (Hurd et al. 2007). The wasps are polyphagous, and therefore their diets are not limited to flower nectar and fruit pulp (Harris 1991). *Vespula germanica* also hunt and consume a variety of arthropods (Kasper et al. 2004). Soft bodied insects are chewed up and returned to the nest where they are fed to the larvae. Each nest, containing hexagonal cells, is started in spring months by a single queen who rears initial workers. Each cell houses a wasp through its developmental stages; egg, five larval instars, and pupae (Kasper et al. 2008). Once the workers are fully grown, they continue to expand the nest and rear more workers during the summer months. Toward the end of the season, colony growth slows and more males are produced (Archer 1997). Smaller cells then begin to be replaced by larger cells which will house new queens and additional males (Kasper et al. 2008). At the start of autumn, the new mated queens disperse and hibernate during the winter months. The colony then disintegrates once the old queen dies. There have been instances in *Vespula* spp. in which the nests continue over winter, forming very large polygynous nests of related sister foundresses (Spradbery and Maywald 1992; Goodisman et al. 2001; Kasper et al. 2008).

### 1.3.3. *Vespula germanica* as an invasive species

*Vespula* wasps are renowned invasive species that have successfully established in several countries (Lester and Beggs 2019). *Vespula germanica* is one notorious example of invasive dominance. This species is native to the Palearctic region, its distribution encompasses Europe up to 62°N, the Mediterranean including Algeria and Morocco, and eastwards to northern India, China and Korea to 23°N (Spradbery and Maywald 1992). However, due to accidental human transport it has since established itself in Australia, New Zealand, North America, South Africa, and South America (Thomas 1960; Archer 1998; Sackmann et al. 2001; D'adamo et al. 2002; Beggs et al. 2011; de Villiers et al. 2017). Due to their eusocial and polyandrous nature, the queen hibernation behaviour and the ability to store sperm from multiple male matings has proven to be advantageous to their survival during transport in cargo across the world (Spradbery and Maywald 1992). The New

Zealand invasion of *V. germanica* is believed to have established in 1945, when military equipment crates containing hibernating queens were flown to Hamilton from Europe (Thomas 1960). The first discovery of the wasp in Australia was in 1959 on the island of Tasmania and accidental transport between cities increased their spread (Crosland 1991). The German wasp invasion to Argentina in the 1980's (Beggs et al. 2011) is thought to have arrived from a population along the French and Spanish border (Brenton-Rule et al. 2018). This result was not surprising considering the historical trade between Argentina and Spain/France. Most *Vespula* species mate multiple times obligatorily in their native and invasive ranges (Loope et al. 2014). This is the case for *V. germanica* in their native range (Bonckaert et al. 2008) and in an introduced population in Australia (Goodisman et al. 2002).

The invasion of *V. germanica* has had numerous impacts in their introduced ranges both economically and health-wise (Lester and Beggs 2019). Studies have been conducted to estimate the impacts of the *Vespula* spp. invasion in New Zealand and has revealed a NZ\$133 million cost to the country (Macintyre and Hellstrom 2015). Invasions in other regions have resulted in negative impacts on apiculture, livestock and the arable sector (Lester and Beggs 2019).

#### **1.3.4. *Vespula germanica* in South Africa**

Overall in South Africa, biological invasions have been calculated to cost around ZAR 9 billion annually (van Wilgen and Lange 2011). The Cape Floristic Region of South Africa (Fynbos biome) has an exceptionally high level of plant endemism (Myers et al. 2000). The indigenous plant species richness in this region has been reduced by the introduction of invasive plants (Richardson et al. 1989). It is therefore of great concern that *V. germanica* (and other invasive invertebrates) have also established in this region (Giliomee 2011). The wasp was first recorded in South Africa in 1974, where it was identified at the South African Museum in Cape Town (Whitehead and Prins 1975). Unpublished evidence has been recorded prior to this, in which a mutilated specimen was brought to the museum for identification in 1972 by pest controllers (Whitehead and Prins 1975). The specimen from 1974 was found in a nest located near a container depot in Irene. This location suggests a possible mode of entry into the country (Tribe and Richardson 1994).

*Vespula germanica* is currently only documented in the Western Cape region of South Africa (Veldtman et al. 2012; de Villiers et al. 2017). This wasp is known to have economic,

social and environmental impacts in this invaded range (Haupt 2015), where it has been known to attack bee hives directly and damage fruit in the apiculture and agricultural sectors. Due to the aggressive nature of the wasps, farm-workers have been less productive when grape-picking in the vineyards (Haupt 2015). The wasp has been confined to a small region of South Africa, however further spread to more suitable eco-climatic conditions has been suggested by some authors to be likely (Spradbery and Maywald 1992; Tribe and Richardson 1994). Studies conducted on the influence of climate on population dynamics of the common wasp lends to support this hypothesis (Lester et al. 2017). Recent modelling studies conducted in South Africa further reveal that *V. germanica* has the potential to expand to more suitable niches occupying a vast majority of the Eastern part of the South Africa (de Villiers et al. 2017). There has been, however, little genetic research on *V. germanica* in South Africa since its initial introduction in Cape Town (Whitehead and Prins 1975; Veldtman et al. 2012).

#### **1.4. Genetics in invasion success**

Population genetics has become a useful tool in the investigation of invasive populations. It provides background information to the invasion process and assists in deciphering invasion colonization processes and demographics (Sakai et al. 2001). This background knowledge of the invasive process is important as it assists in the design of mitigation strategies to prevent further spread (Sakai et al. 2001; Beggs et al. 2011; Masciocchi and Corley 2013; Brenton-Rule et al. 2018). Geographical data alone is an inadequate means to determine scale of eradication as there are multiple additional factors that influence invasion success including dispersal abilities, reproductive capabilities, enemy presence and genetic variation (Moller 1996). Further investigation as to the role of genetic variation in the invasion process is usually needed. For example, a loss of genetic diversity could be compensated for by phenotypic plasticity and could be crucial in the success of biological invasions (Bock et al. 2015).

During the initial colonization of introduced species, a typical introduction will consist of a small number of individuals. Due to this small number of individuals, a genetic bottleneck may occur (Ellstrand and Elam 1993; Sakai et al. 2001). In order for a population to successfully persist and expand, the invasive species must overcome this bottleneck. Genetic variation has been observed to influence fitness in introduced ranges

(Vandewoestijne et al. 2008; Markert et al. 2010; Dobelmann et al. 2017). The lack of genetic variation can result in inbreeding depression which can limit the populations' adaptive evolution capabilities and in turn limit population growth (Ellstrand and Elam 1993; Nieminen et al. 2001). In some cases, the genetic constraints present after a bottleneck could also positively influence the rate of expansion through novel selective regimes as observed in invasive populations of the Argentine ant (*Linepithema humile*) (Tsutsui et al. 2000). Another case is observed in the invasion of *A. cerana* into Australia, the haplo-diploid sex determination system was expected to exacerbate their susceptibility to a founder effect. However, negative frequency-dependant selection occurred in which rare *csd* (complementary sex determiner) alleles promoting viable offspring were favoured. The favouring of these *csd* alleles, balanced selection and ultimately facilitated their invasive success (Gloag et al. 2017).

Genetic variation is a key component in adaptive evolution. Genetically diverse populations of invasive species are typically more prevalent when multiple introductions have occurred (Sakai et al. 2001; Dlugosch and Parker 2008; Garnas et al. 2016). This increase in diversity results in an increase in the adaptive evolution capacity (Garnas et al. 2016). Some phenotypical changes due to genetic variation may encourage invasiveness, whereas some variations may be deleterious (Marsden et al. 2016). Harmful alleles that reduce the fitness of new populations can be removed through founder effect (Garnas et al. 2016). Gene flow between populations from multiple introductions could exacerbate the spread of beneficial characteristics by introducing variation that could lead to further adaptation under new environmental conditions (Bock et al. 2015; Marsden et al. 2016). In summary, an examination of the genetics of invasive populations can assist in the understanding of the spread of an invasive species and in its future conservation and management (Estoup and Guillemaud 2010).

### **1.5. Use of molecular markers in invasion studies**

Molecular biology advancements has led to the development of various molecular markers (Schlötterer 2004) including microsatellites, maternally inherited mitochondrial DNA (mtDNA), or bi-parentally inherited Single Nucleotide Polymorphisms (SNP's). Studies using DNA molecular markers can determine whether multiple introductions have occurred and give an estimate of the genetic variation present after an introduction event. Brenton-

Rule et al. (2018) examined mitochondrial DNA (mtDNA) variation in the native and invaded ranges of *V. germanica* to determine source of origin and intra-specific variation. Most of the invasions in the Southern Hemisphere appeared to originate from multiple introductions. A comparison of haplotypes from the invasive and native ranges of this wasp found haplotype matches suggesting where the source of origin of those invasions were, with the exception of South Africa. None of the mtDNA haplotypes surveyed from the South African populations of *V. germanica* matched a previously sampled haplotype from the native range. The most likely origin of this invasion was suggested to be mainland Europe (Brenton-Rule et al. 2018). A more recent study that examined the nuclear genetic structure and diversity of *V. germanica* and *V. vulgaris* included three *V. germanica* individuals from South Africa. The results suggested that the South African *V. germanica* population originated in mainland Europe in agreement with the mtDNA data (Schmack et al. 2019); however, further sampling is needed to narrow down the source population. Two of the more commonly used molecular markers (mtDNA and microsatellites) in population genetic studies are described below.

### **1.5.1. Mitochondrial DNA**

Mitochondrial DNA is a small, double-stranded, circular and haploid molecule, that due to its unique characteristics has increasingly become used as a molecular marker in determining origin of invasions and phylogenetic relationships in invasion biology (Lester et al. 2014; Barral-Arca et al. 2016). The mtDNA codes for proteins used in gene transcription and respiration of the cells (Benkhalifa et al. 2014) (Figure 2). It is also maternally inherited and non-recombinant with high nucleotide substitution rates (Brown et al. 1979). Variations in the mitochondrial genome has been linked to phenotypical changes, affecting reproduction, energy metabolism and ageing (Dowling et al. 2008). How these effects influence the organism is dependent on the relationship between the mitochondrial variant and the nuclear genome as these two genomes interact and coevolve with one another (Dowling et al. 2008). These characteristics as well as its easy isolation and high mutation rate make it a viable marker in population genetics (Desalle et al. 2017).

In *Vespula germanica*, the mitochondrial genome is around 16,342 bp in length (Zhou et al. 2016). The circular mitochondrial genome of *V. germanica* and the location of the PCR and Sanger sequencing primers used in population genetic studies are shown in Figure 2. These primers have previously been used in genetic studies of *V. germanica* (Brenton-Rule

et al. 2018; Dobelmann et al. 2019). In the study conducted by Brenton-rule et al. (2018), the mtDNA phylogenetic relationship and genetic diversity of *V. germanica* was examined in its home and invaded ranges. The study was able to identify most of the haplotype diversity in Argentina, New Zealand, and Australia. The samples from the native range (United Kingdom and Europe), were suggested to have haplotypes that were not sampled. The full haplotype diversity in South Africa was also indicated as not being sampled (Brenton-Rule et al. 2018). Increasing sample numbers could sample the full mitochondrial diversity present in South Africa. A comparison of haplotypes from the invasive and native ranges of this wasp found haplotype matches suggesting where the source of origin of those invasions were, with the exception of South Africa (Brenton-Rule et al. 2018). None of the mtDNA haplotypes surveyed from the South African populations of *V. germanica* matched a known haplotype from part of the wasp native range in Europe (Brenton-Rule et al. 2018). The objectives of our study were to estimate how many successful *V. germanica* wasp establishments have occurred in South Africa using mtDNA. For the purpose of this study we considered that each individual haplotype that we observed likely represents a single introduction event, though we recognise that one haplotype may have been introduced on multiple occasions.



utilizing microsatellites has revealed *V. germanica* as a polyandrous species, in which a single queen has multiple male mates (Goodisman et al. 2002). Despite the potential presence of null alleles, microsatellites are still classed overall as a good neutral mendelian marker in population genetic analyses (Jarne and Lagoda 1996). Null alleles can be described as a non-functional mutation type, that results in a lack of gene product or function at the phenotypic level (Snustad and Simmons 2012). Neutral mendelian marker refers to a measurable mendelian trait that is inherited but not adaptive (neutral).

We sought to establish if there is population structure among *V. germanica* sampled from different localities in South Africa, using microsatellites. High diversity among locations would be indicative of population differentiation and limited dispersal, whereas a lack of genetic structure would suggest a homogeneous population with movement of individuals between localities.

## 1.6. Framework and Objectives

Using mitochondrial DNA and microsatellites as molecular markers, this thesis aims to fill in the gaps with regard to the population genetics of *V. germanica* in South Africa through the following objectives: 1) Estimate how many successful *V. germanica* wasp establishments have occurred in South Africa using mtDNA. Additional introductions with different haplotypes may have been present but were unable to persist past the initial colonisation stage, and thus only successful introductions leading to establishments can be determined; 2) Establish if there is population structure and differentiation among *V. germanica* sampling locations in South Africa; 3) Determine the origin of the South African *V. germanica* population using mtDNA and microsatellites.

We hypothesize that there will be multiple introductions that have occurred in the South African *V. germanica* population, based on the dynamics of invasions in other regions (Brenton-rule et al. 2018). We hypothesize that population genetic structure will be reduced following a genetic bottleneck based on invasion dynamics. We hypothesize that the invasion originated from a location within mainland Europe, as per suggestions by Schmack et al. (2019) and Brenton-Rule et al. (2018).

This work will provide insight into the invasion genetics of *V. germanica* in South Africa and potentially assist in the future design of mitigation strategies.

## 2. Materials and Methods

### 2.1. Sample collection

*Vespula germanica* wasps were collected from 42 nests across the invaded range in South Africa from 2016-2019 (Figure 3). We extracted DNA from a total of 62 worker wasps from the 42 nests (20 wasps were replicates from some of the nests genotyped to ensure consistency but not included in the analyses as nestmates are related and not independent from one another; (Goodisman et al. 2001) (Supplementary Table 1). All samples were immediately preserved in 99% ethanol and frozen at -20°C once received in the laboratory.

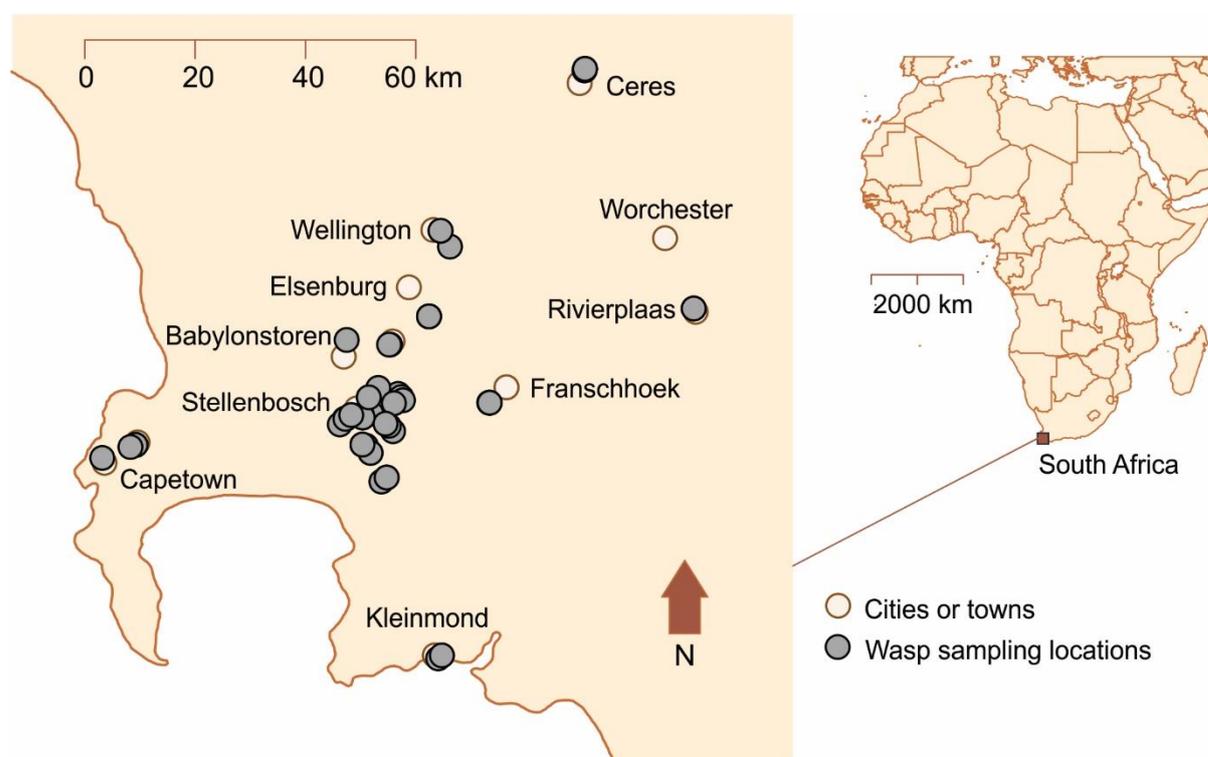


Figure 3. Map showing the *Vespula germanica* sample locations across the invaded range in South Africa.

Five individuals from mainland Europe were also included from Spain, Portugal, Austria, Belgium and France for the mtDNA analyses. Mainland Europe was selected as it is the suggested source of the *V. germanica* introduction into South Africa (Brenton-Rule et al. 2018). We also incorporated microsatellite data for European *V. germanica* samples from Schmack et al. (2019) for comparison.

## 2.2. DNA Extraction

DNA was extracted from individual wasps by homogenizing the entire wasp, using a combination of GENEzol reagent (Geneaid, Taiwan) and 5%  $\beta$ -mercaptoethanol following procedures used in Brenton-Rule et al. (2018). The extraction was then purified using standard chloroform/ isopropanol protocols.

The extraction process involved adding one whole wasp into a 2 ml cryotube between two 6 mm metal beads. 1 ml GENEzol reagent (Geneaid, Taiwan) as well as 50  $\mu$ l  $\beta$ -mercaptoethanol was added to the cryotube. The tube was then placed in the Precellys Evolution homogenizer (Bertin, France) on 'hard' settings (6300 rpm, for 2 x 20 second cycles, with a 30 second pause after each cycle) for 1 minute to break down the exoskeleton of the wasp. Once homogenized, the tube was placed in a heat block at 65°C for 10-15 minutes, shaking frequently. The liquid was then separated from the exoskeleton manually using a pipette and placed into a 2 ml centrifuge tube. The centrifuge tube was then placed back into the 65°C heat block for a further 10 minutes. The tube was then centrifuged at 14 000 rpm for 5 minutes. Once centrifuged, 650  $\mu$ l of the supernatant (top layer) was added to a new 2 ml tube containing 650  $\mu$ l chloroform/ IAA to begin the purification steps. The tube was then vortexed until a white emulsion was visible prior to being centrifuged for 5 minutes, creating layer separation. 500  $\mu$ l of the top layer was then added to a tube containing 350  $\mu$ l of cold isopropanol. The tube was then inverted 10 times to mix the extraction prior to centrifuging for a further 10 minutes at 14 000 rpm. A DNA pellet then formed at the bottom of the tube and the remaining liquid was poured out carefully, ensuring that the pellet stayed in place. 300  $\mu$ l of 70% ethanol was then added to the tube. The tube was then briefly vortexed and centrifuged for 5 minutes at 14 000 rpm. Once centrifuged, the ethanol was poured out and the pellet left to dry in the tube on a paper towel at room temperature for 30 minutes. The pellet was then re-suspended by adding 100  $\mu$ l of chilled MQ water and inverted. The final extraction was then aliquoted into 2 separate cryotubes, each containing 50  $\mu$ l of the extracted DNA (one for back-up storage at -80°C and one for general use).

The DNA concentrations of each individual were determined using a NanoPhotometer (Implen, Germany) (Supplementary Table 2). Only samples with DNA concentrations above 50 ng/ $\mu$ l were used for the analysis. Samples with DNA concentrations above 500 ng/ $\mu$ l were diluted 1:2 with 50  $\mu$ l MQ H<sub>2</sub>O. This procedure was to ensure that excessive

amounts of DNA were not present when conducting PCRs as a large quantity of DNA in the PCR may lead to an unbalanced amplification of loci/alleles.

### 2.3. Mitochondrial DNA analysis

Polymerase chain reaction (PCR) was used to amplify portions of *cytochrome b* (*cytb*), *cytochrome c oxidase I* (*COI*) and *cytochrome c oxidase II* (*CO2*) genes. *CO2* incorporates the intergenic region between *CO2* and *COI* that codes for tRNA Leu. These loci were selected as they were informative for *V. germanica* in previous work (Brenton-Rule et al. 2018; Dobelmann et al. 2019). Primer sequences and their sizes are listed in Table 1. Each 25 µl PCR reaction mix comprised 1 µl template DNA between 50-500 ng/µl, 1 µl each of 10 nM forward and reverse primers (Invitrogen, Thermo Fisher Scientific), 9.5 µl of milliQ H<sub>2</sub>O and 12.5 µl MyTaq Mix (Bioline, London, UK). The PCR cycling conditions for each mitochondrial gene are shown in Table 2. PCR products were then visualised using gel electrophoresis. Agarose gel electrophoresis is generally used to separate DNA by size. In this case it was used as a preliminary step to see whether the PCR had worked prior to sending samples for Sanger sequencing (Figure 4). The 1.5 % agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, Thermo Fisher Scientific) was run at 80 V for 30 minutes in a 1,5 x TBE Buffer with each well containing 1 µl of the PCR product mixed with 1 µl BlueJuice Gel Loading Buffer (10 x) (Invitrogen, Thermo Fisher Scientific). Gel pictures were captured using an Essential V6 UV transilluminator (Uvitec, Cambridge).

A PCR was rerun for individuals if no DNA band was visible in the agarose gel under UV light. The PCR product was then purified using ExoSAP-IT Express (Affymetrix Thermo Fisher Scientific). This is done by mixing 5 µl of the PCR product with 2 µl of ExoSap-IT Express reagent and then gently vortexing the mix prior to a brief centrifuge to bring the contents to the bottom of the tube. The tube is then placed in a thermal cycler and set to incubate at 37°C for 4 minutes, followed by an incubation of 80°C for 1 minute to inactivate the ExoSap-IT Express reagent, and then held at 4°C prior to being transferred to ice. In order to prepare the sample for sequencing, 2 µl of the cleaned PCR product was suspended in 5.5 µl MQ H<sub>2</sub>O and 2.5 µl of the forward primer. Unidirectional Sanger sequencing was performed on an ABI 3730xl DNA Sequencer (Macrogen, Seoul, Republic of Korea). Sequences were manually trimmed in Geneious 8.0.5 (Kearse et al. 2012). The ends of each of the sequences per gene were trimmed prior to being concatenated for analysis, so as to

ensure only sequences and base calling of high quality was used (A total of 1518 bp was used for the concatenated analysis post trimming) (Supplemental Figure 1). The sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) in Geneious 8.0.5 (Kearse et al. 2012). All sequences have been placed on GenBank with accession numbers: MN727980-MN728042, MN736210-MN736272 and MN736273-MN736335. See Supplementary Table 1 for detailed accession number information relating loci with specimens. Sequences were verified using NCBI BLASTn search (<http://blast.ncbi.nlm.nih.gov/>) to confirm the sequence authenticity of the samples as *V. germanica*. Sequences of one nest was excluded due to their low quality, thus a total of 41 nests were included in the mtDNA analyses. Mitochondrial DNA analyses also included five individuals, one each from Spain, Portugal, Austria, Belgium and France, respectively from *V. germanica*'s native range in mainland Europe.

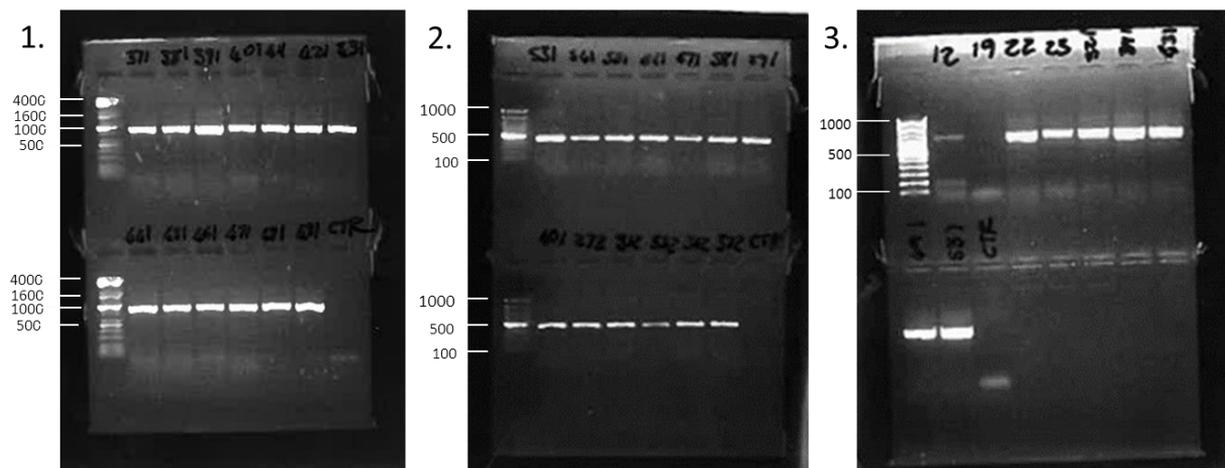


Figure 4. Electrophoresis gel examples conducted in this study to visualise PCR products of individuals for: 1.CO1 gene, 2.Cytb gene and 3. CO2 gene. The columns on the far left in gel 1 contain the KAPA universal ladder used to compare size. A 100bp DNA ladder was used for the Cytb and CO2 gels respectively. The size (bp) is shown on the left of each gel. Each gel was run in 1.5% agarose in a 1.5 x TBE buffer. Gel pictures were visualised using an Essential V6 UV transilluminator (Uvitec, Cambridge, UK).

Table 1. PCR primers used to amplify mtDNA in this study (Simon et al. 1994; Dobelmann et al. 2019). The forward primer is listed first, followed by the reverse primer.

Target gene	Primer name	Sequence 5'-3'	Amplicon (bp)	Reference
Cytochrome b	CB10933	TATGTACTACCATGAGGACAAA TATC	473	Simon et al. (1994)
	CB11367	ATTACACCTCCTAATTTATTAG GAAT		
Cytochrome oxidase 1 (CO1)	Vg Cox1b F	TACCAGTTCTTGCAGGAGCAAT	940	Dobelmann et al. (2019)
	Vg Cox1b R	GTGGCGTAAGGAATTTGTTCA		
Cytochrome oxidase 2 (CO2)	Vg tLeuC F	ATCTGGTTTTCTCGACGATAC T	854	Dobelmann et al. (2019)
	Vg tLeuA R	TACGTCCAGGGGTAGCATCA		

Table 2. PCR cycling conditions for cytochrome b (cytb), cytochrome oxidase I (CO1), and the intergenic region between cytochrome oxidase subunit II and CO1 (CO2).

	Cytb			COI			CO2		
	Temperature (°C)	Time	Number of cycles	Temperature (°C)	Time	Number of cycles	Temperature (°C)	Time	Number of cycles
Initial denaturation	94	2 min		95	1 min		95	1 min	
Denaturation	94	30 sec		95	15 sec		95	30 sec	
Annealing	45	40 sec	40	47	15 sec	35	47	30 sec	40
Extension	72	1 min		72	1 min		72	55 sec	
Final extension	72	10 min		72	5 min		72	5 min	

### 2.3.1. Phylogenetic analysis

For the phylogenetic analysis we concatenated the three mtDNA genes (1,518 base pairs) and homologous sequences for one *Vespula vulgaris* individual were downloaded from GenBank to be used as the outgroup. The most suitable model of sequence evolution was determined using BIC (Bayesian Information Criterion) scores in MEGA7 (Kumar et al. 2016). A phylogenetic tree was inferred using the MrBayes v.3.2.6 (Huelsenbeck and Ronquist, 2001) plug-in for Geneious. Clade probabilities were obtained from the posterior distribution. Bayesian analyses were replicated twice, each with four Markov chains of 2 million generations. Trees were sampled every 2500 generations, of which the first 0.5 million generations were discarded as burnin.

### 2.3.2. Haplotype network

A mitochondrial haplotype network of the South African and five European samples was inferred using the median-joining algorithm in NETWORK v.5 (<http://www.fluxus-engineering.com>; Bandelt et al. 1999). A haplotype accumulation curve was constructed using the R package *vegan* (Oksanen et al. 2019). This curve was used to determine if the full haplotype diversity of South African wasps sampled (Oksanen et al. 2019). Should the curve have reached a horizontal asymptote, then it can be inferred that the haplotype diversity has been sampled adequately.

### 2.4. Microsatellite Analysis

Thirteen microsatellite loci previously designed for *Vespula rufa* (Thorén et al. 1995; Foster et al. 2001), *Dolichovespula* spp., *Vespa cabro* and *Vespula* spp. (Daly et al. 2002), *Vespa mandarinia* (Hasegawa and Takahasi 2002) and *Vespa velutina* (Arca et al. 2012) were assayed. Of these 13, ten loci amplified successfully (Table 3). We genotyped these ten loci for each of the 62 *V. germanica* individuals from South Africa following methods used in Schmack et al (2019). At the 5'-end of each forward primer, a M13-tag (TGTAACGACGGCCAGT) was added. Each of the 10 loci were amplified in a 10µl PCR reaction per individual containing 5 µl MyTaq Mix (Bioline, London, UK), 0.2 µl of 10 nM forward primer, 0.8 µl of 10 nM reverse primer, 0.8 µl M13-primer (labelled with one of four dyes; 6-FAM, HEX, PET, NED), 0.8 µl 10 nM Bovine Serum Albumin (Sigma Aldrich), 1.4 µl milliQ H<sub>2</sub>O, and 1 µl of the template DNA (with concentrations 50-500 ng/µl). Each locus was assigned a specific dye and where possible completed in a single run to avoid dye shifts (Sutton et al. 2011).

PCR thermal cycling conditions, primer annealing temperatures and mixture groups are included in Tables 3 and 4. PCR products were visualised using agarose gel electrophoresis. The 3 % agarose gel (TAE) stained with SYBR Safe DNA gel stain (Invitrogen, Thermo Fisher Scientific) was run at 80 V for 30 minutes in a 3 x TAE Buffer with each well containing 1 µl of the PCR product mixed with 1 µl BlueJuice Gel Loading Buffer (10 x) (Invitrogen, Thermo Fisher Scientific). Gel pictures were captured using an Essential V6 UV transilluminator (Uvitec, Cambridge)(Figure 5). This visualisation was done to ensure the PCR had worked prior to sending the PCR products for genotyping. Genotyping was

performed using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at Massey Genome Service (Massey University, Palmerston North, New Zealand). Alleles were sized using GeneScan 500 LIZ (Applied Biosystems) ladder and scored by hand using Geneious 8.0.5 (Kearse et al. 2012) (Supplemental Figure 4). The allelic scores obtained for the South African *V. germanica* wasps in this study are presented in Supplementary Table 3. Nine of the 10 amplified loci were variable and included in further analysis. To assess any possible scoring errors that may have occurred due to the presence of null alleles, stuttering and allelic dropout we used Microchecker v2.2.3 (Van Oosterhout et al. 2004)

Table 3. Locus name, PCR annealing temperatures (Ta), and sources of microsatellite loci assayed on *V. germanica*. \*Locus failed to amplify (0), locus presented excessive stuttering (s), locus amplified but not variable (y) and locus PCR-amplified and was variable (+)

<b>Locus</b>	<b>Developed for</b>	<b><i>V. germanica</i> Outcome*</b>	<b>Ta (°C)</b>	<b>Cycling conditions</b>	<b>Source</b>
<b>Rufa 5</b>	<i>Vespula rufa</i>	+	55	33 cycles	Thoren et al. (1995), Foster et al. (2001)
<b>Rufa 15</b>	<i>Vespula rufa</i>	0	55.5	33 cycles	Thoren et al. (1995), Foster et al. (2001)
<b>Rufa 18</b>	<i>Vespula rufa</i>	0	52	33 cycles	Thoren et al. (1995), Foster et al. (2001)
<b>Rufa 19</b>	<i>Vespula rufa</i>	+	55	33 cycles	Thoren et al. (1995), Foster et al. (2001)
<b>LIST2003</b>	Eusocial wasps (Vespidae)	s	55	33 cycles	Daly et al. (2002)
<b>LIST2004</b>	Eusocial wasps (Vespidae)	+	55	33 cycles	Daly et al. (2002)
<b>LIST2007</b>	Eusocial wasps (Vespidae)	+	55	33 cycles	Daly et al. (2002)
<b>LIST2011</b>	Eusocial wasps (Vespidae)	+	55	33 cycles	Daly et al. (2002)
<b>LIST2019</b>	Eusocial wasps (Vespidae)	s	55	33 cycles	Daly et al. (2002)
<b>VMA-6</b>	<i>Vespa mandarinia</i>	+	56	33 cycles	Hasegawa & Takahasi (2002)
<b>VMA-7</b>	<i>Vespa mandarinia</i>	+	58	33 cycles	Hasegawa & Takahasi (2002)
<b>R4-114</b>	<i>Vespa velutina</i>	+	55	45 cycles	Arca et al. (2012)
<b>D2-142</b>	<i>Vespa velutina</i>	0	55	46 cycles	Arca et al. (2012)

## References

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- Thorén PA, Paxton RJ, and Estoup A (1995) Unusually high frequency of (CT)<sub>n</sub> and (GT)<sub>n</sub> microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). *Insect Molecular Biology*, 4, 141–148.

Table 4 Multiplex PCR groups, dyes and allele sizes for *Vespula germanica* in this study.

	<b>Locus</b>	<b>Dye</b>	<b>Allele size range</b>
<b>Mix 1</b>	LIST2007	6-FAM	176-185
	VMA-7	HEX	156-160
	VMA-6	PET	293-322
	LIST2004	NED	159-186
<b>Mix 2</b>	Rufa 5	6-FAM	159-175
	Rufa 19	HEX	212-226
	R4-114	PET	131-135
	LIST2011	NED	143-146
<b>Mix 3</b>	LIST2019	6-FAM	161-169
	LIST2003	HEX	238-244

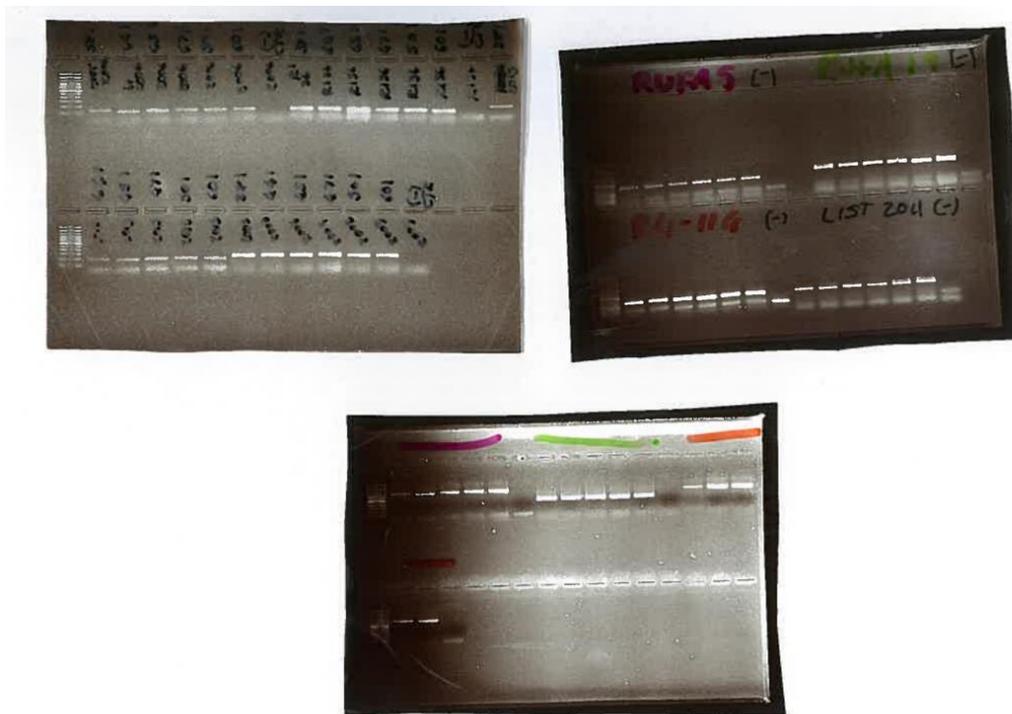


Figure 5 Agarose gel electrophoresis examples conducted in this study to visualise PCR products of individuals for each microsatellite mix. The top left represents mix 1, top right is mix 2 and the bottom gel represents mix 3 as well as re-dos for R4-114 and Rufa 19 (each loci was represented by a coloured band). Each gel was run in 3% agarose in a 3 x TBE buffer. Gel pictures were visualised using an Essential V6 UV transilluminator (Uvitec, Cambridge, UK).

### 2.4.1. Analysis of genetic diversity

The South African wasp population descriptive statistics were calculated using GenAlEx v.6.5 (Peakall and Smouse 2012). The number of alleles ( $N_a$ ) was determined by direct count. The observed heterozygosity ( $H_o$ ) where the number of heterozygotes was determined by N through direct count; and the expected heterozygosity ( $H_e$ ) was determined per locus as the sum of the squared allele frequencies ( $p_i^2$ ) subtracted from 1.

$$H_o = \frac{\text{No. of Hets}}{N} \quad H_e = 1 - \sum p_i^2$$

We calculated genetic diversity and allelic richness per locus and population using FSTAT v.2.9.3 (Goudet 1995). Potential deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were estimated with Genepop v.4.2 (Rousset 2008) with Markov chain parameters: 1,000 dememorizations, 1,000 batches, and 10,000 iterations per batch. Significance levels ( $p = 0.05$ ) for departure from HWE and LD were corrected for multiple comparisons.

### 2.4.2. Analysis of genetic structure

To designate individuals into admixture proportions based on their allele frequencies, we used the program STRUCTURE v.2.3.4 (Pritchard et al. 2000). The dataset was analysed applying the admixture model with the assumption of correlated frequencies, 100,000 run burn-in period, 1,000,000 Markov Chain Monte Carlo iterations, and the possible number of South African *V. germanica* populations ( $k$ ) ranged from 1 to 7. The analysis was repeated ten times across runs in order to ensure consistency. The Evanno et al. (2005) method was used to determine the most plausible number of clusters in our dataset as implemented in STRUCTURE HARVESTER web v.0.6.94 (Earl and vonHoldt 2012). We also re-run the STRUCTURE analysis as described above including our samples, plus 20 European and three additional South African wasps genotyped by Schmack et al. (2019). Allelic scores from Schmack et al. (2019) were matched to ours by hand, as both studies were conducted in the same laboratory. Therefore, for the second STRUCTURE run we only included the seven loci in our study (Rufa 5, Rufa 19, List 2004, List 2007, List 2011, List 2019, R4-114) that were also genotyped by Schmack et al. (2019). Sample sizes were  $n = 45$  for South Africa and  $n = 20$  for Europe.

We carried out a factorial correspondence analysis (FCA) of multilocus genotypes to visually represent genetic variation at the individual level with GENETIX v.4.05 (Belkhir et al. 1998). Factorial correspondence analysis are computed in the bivariate space defined by the first two factorial components. The FCA incorporated data of *V. germanica* from Europe ( $n = 20$ ) from Schmack et al. (2019). The overall FCA sample sizes were 45 South African nests, and 20 European wasp nests.

### 3. Results

#### 3.1. Number of successful wasp establishments in South Africa

The mitochondrial data showed six unique haplotypes in our dataset from South Africa (H1– H6). Two haplotypes were more common; H1 was the most common haplotype, found in 31 wasps from South Africa and one wasp from Austria. The second most common South African haplotype (H2) was represented by five wasps from five different locations. These two haplotypes were three nucleotide substitutions apart. Two samples from Jonkershoek share the same haplotype (H3) and the remaining three haplotypes were represented by an individual sample from Kleinmond (H4), Worcester (H5) and Stellenbosch (H6), respectively (Figure 6). Truncated aligned sequences are shown in Supplementary Figure 2, indicating the polymorphic regions and positions where SNPs occur.

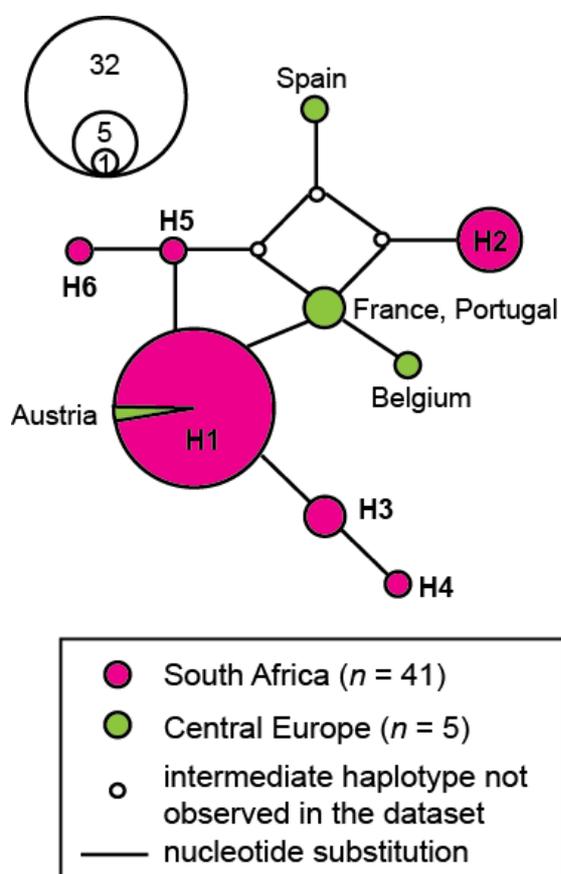


Figure 6. Haplotype network of South African and European *V. germanica* samples. Each of the circles represent a unique haplotype represented by H1-H5. The size of the circle correlates to the number of individuals. The largest haplotype contained 31 individuals, followed by the second largest haplotype with 5 individuals.

The rarefaction analysis showed that the haplotype discovery curve is beginning to plateau, indicating that much of the South African genetic diversity has been sampled in our study (Figure 7). It is possible, however, that additional genetic diversity could be found with additional sampling.

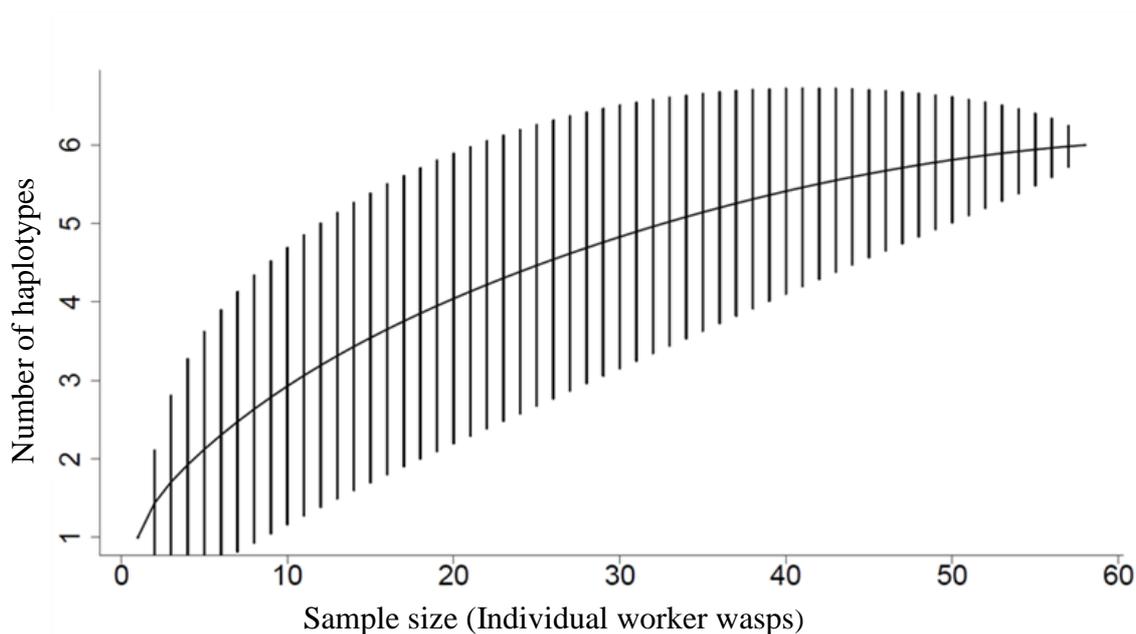


Figure 7. Haplotype accumulation curve showing the six haplotypes sampled in South Africa. The vertical lines represent 95% confidence intervals.

The best-fitting model of sequence evolution was determined to be the Hasegawa-Kishino-Yano (HKY) model, which was then used to construct the phylogenetic tree (Hasegawa et al. 1985); Table 5). The Bayesian phylogeny shows that all six haplotypes sampled are closely related. The number of nucleotide substitutions between haplotypes varied from one to five (Figure 8). The percentage of sequence similarity for the 1,518 bp dataset was 99.5% including the five European samples.

1 Table 5. Top five maximum likelihood fits of the 24 different nucleotide substitution models as calculated in MEGA7 for the concatenated Cytb, CO1 and CO2 datasets (1518  
 2 bp). Model abbreviations: HKY= Hasegawa-Kishino-Yano; TN93= Tamura-Nei; T92= Tamura 3-parameter. The best-fitting model was based on the lowest Bayesian  
 3 Information Criterion (BIC) score. The Maximum Likelihood value (InL) is also shown. The substitution is best described by models presenting the lowest BIC. Where  
 4 applicable the estimates of +G (Gamma distribution) and +I (invariable sites) are shown. Estimated values of transition/transversion bias ( R) is shown for each model.

<b>Model</b>	<b># parameters in the model</b>	<b>BIC</b>	<b>InL</b>	<b>(+I)</b>	<b>(+G)</b>	<b>Transition/transversion bias (R)</b>
HKY	127	5388.902	-1966.21	-	-	4
TN93	128	5390.482	-1961.27	-	-	4.01
T92	125	5393.767	-1980.11	-	-	4
HKY+G	128	5394.201	-1963.13	-	0.05	4.04
TN93+G	129	5395.751	-1958.17	-	0.05	4.08

5

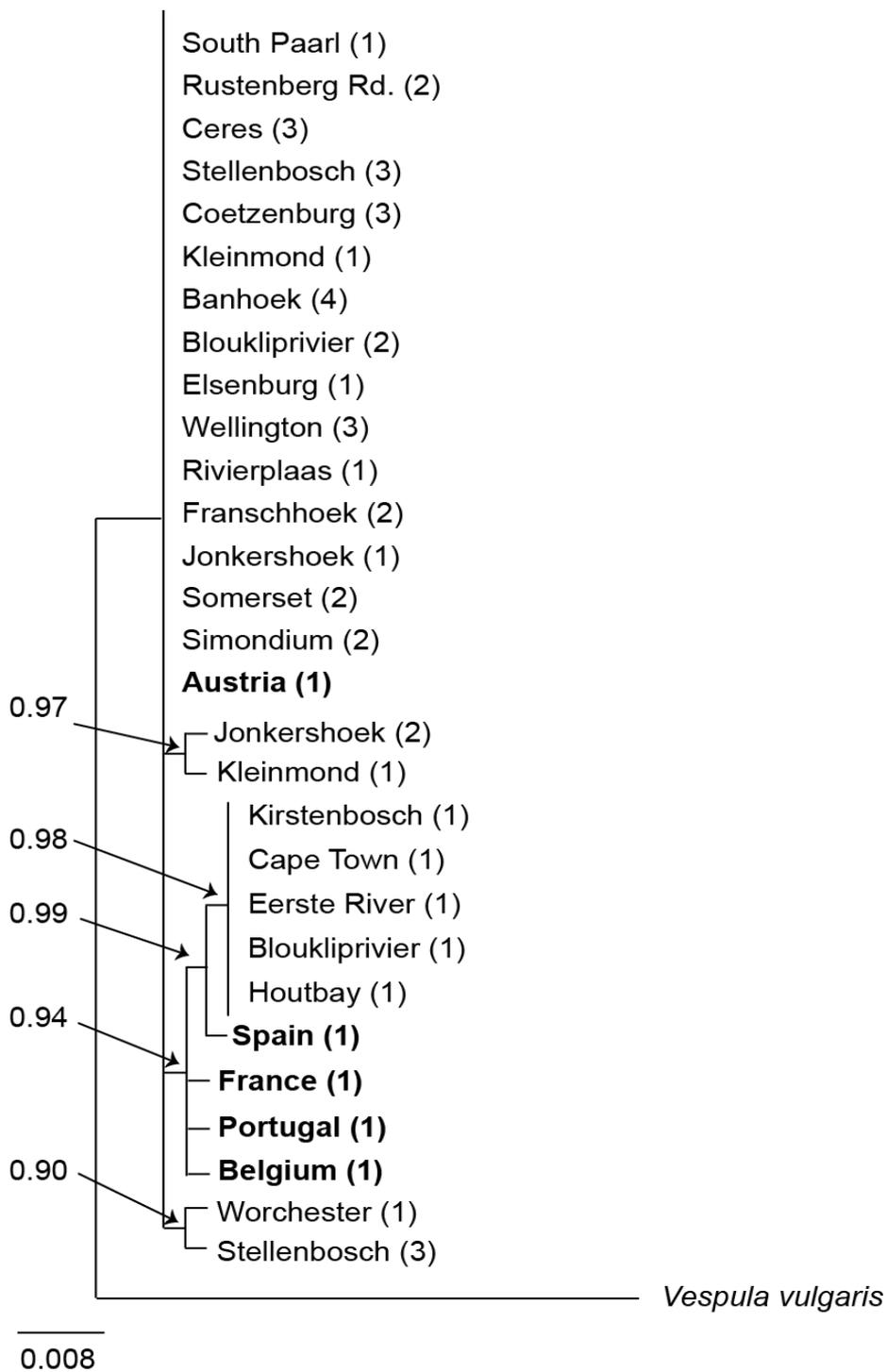


Figure 8. Maximum composite likelihood tree for *V. germanica* in South Africa (n = 41) and mainland Europe (n = 5) drawn from 1,518 bp of concatenated mtDNA data. Number of haplotypes per locality are shown between parentheses. The bootstrap proportion is shown on top of the branches if above 50%.

### 3.2. Mixing and genetic drift in South African wasps

No variation was observed in the microsatellite locus VMA-7 thus, it was excluded in all subsequent analyses. Following sequential Bonferroni correction, no significant linkage disequilibrium was detected among paired loci comparisons. Slight linkage disequilibrium was observed between the loci VMA6 and LIST2004; and LIST2011 and LIST2004 prior to Bonferroni correction (Table 6). Significant deviation from Hardy Weinberg equilibrium was observed for locus LIST2004, following Bonferroni correction. No evidence of scoring errors due to stuttering, null alleles, or large allele dropout were detected in our dataset. We found an average expected ( $H_e$ ) of 0.482 and an observed heterozygosity ( $H_o$ ) of 0.489. The  $H_e$  ranged from 0.024 to 0.661 and  $H_o$  ranged from 0.024 to 0.976 (Table 7). The largest number of alleles was 6, and the smallest number of alleles was 2 (Table 7). The average number of alleles across all loci was 3.333. This small number of effective 266 alleles in South Africa is in agreement with low genetic diversity. When the observed heterozygosity is lower than the expected, it is often attributed to reduced genetic variability (Sharma et al. 2016), which could be a result of a bottleneck, gene flow, inbreeding or the Wahlund effect. The Wahlund effect takes place when two or more subpopulations possess allele frequencies that are different due to genetic drift; this effect further results in a reduction of heterozygosity despite each of the subpopulations being in Hardy-Weinberg equilibrium (Wahlund 2010). When observed heterozygosity is greater than expected, it is often associated with the mixing of previously separate populations.

Table 6. Linkage disequilibrium test results as calculated in Genepop 4.7.0. Bonferroni corrected P-value = 0.0014.

<b>Locus#1</b>	<b>Locus#2</b>	<b>P-Value</b>	<b>S.E.</b>
LIST2007	VMA6	0.5719	0.0010
LIST2007	Rufa5	0.9451	0.0003
VMA6	Rufa5	0.1726	0.0016
LIST2007	Rufa19	0.3310	0.0010
VMA6	Rufa19	0.7454	0.0020
Rufa5	Rufa19	0.8112	0.0013
LIST2007	R4-114	0.6784	0.0004
VMA6	R4-114	0.6244	0.0009
Rufa5	R4-114	0.1332	0.0005
Rufa19	R4-114	0.7576	0.0007
LIST2007	LIST2011	0.1002	0.0003
VMA6	LIST2011	0.0746	0.0004
Rufa5	LIST2011	0.3992	0.0006
Rufa19	LIST2011	0.4506	0.0007
R4-114	LIST2011	0.3223	0.0004
LIST2007	LIST2019	1.0000	0
VMA6	LIST2019	1.0000	0
Rufa5	LIST2019	0.4528	0.0007
Rufa19	LIST2019	0.3807	0.0009
R4-114	LIST2019	0.2140	0.0002
LIST2011	LIST2019	1.0000	0
LIST2007	LIST2003	0.0779	0.0005
VMA6	LIST2003	0.1843	0.0018
Rufa5	LIST2003	0.0698	0.0008
Rufa19	LIST2003	0.2713	0.0019
R4-114	LIST2003	0.1074	0.0006
LIST2011	LIST2003	0.0638	0.0003
LIST2019	LIST2003	0.7378	0.0007
LIST2007	LIST2004	0.1441	0.0006
VMA6	LIST2004	0.0106	0.0003
Rufa5	LIST2004	0.6501	0.0013
Rufa19	LIST2004	0.5826	0.0017
R4-114	LIST2004	0.1924	0.0006
LIST2011	LIST2004	0.0111	0.0001
LIST2019	LIST2004	0.4274	0.0007
LIST2003	LIST2004	0.1594	0.0012

Table 7. Indices of genetic diversity for the invasive *V. germanica* population in South Africa for nine microsatellite loci assayed for 42 individuals representing 42 wasp nests (Thorén et al. 1995; Foster et al. 2001; Daly et al. 2002; Hasegawa and Takahasi 2002; Arca et al. 2012). Data represents the number of alleles ( $N_a$ ), observed heterozygosity ( $H_o$ ), and expected heterozygosity ( $H_e$ ).

	Locus									Overall	
	LIST2007	VMA-6	Rufa 5	Rufa19	R4-114	LIST2011	LIST2019	LIST2003	List2004	MEAN	SE
$N_a$	2	5	4	5	2	2	2	4	4	<b>3.333</b>	<b>0.441</b>
$H_o$	0.333	0.619	0.595	0.595	0.452	0.262	0.024	0.548	0.976	<b>0.489</b>	<b>0.089</b>
$H_e$	0.383	0.624	0.664	0.656	0.489	0.305	0.032	0.649	0.657	<b>0.496</b>	<b>0.073</b>

### 3.1. Genetic structure

The deltaK ( $\Delta k$ ) method suggested that the optimal number of genetic clusters was  $k = 2$ ,  $\Delta k = 7.98$  (Figure 9). After visually inspecting the assignment plot it was clear that the individuals were assigned to each of the two groups relatively equally (Supplementary Figure 2), which indicates that no substructure could be observed based on geography in our dataset. Secondary optima at  $k = 3$ ,  $\Delta k = 1.51$  and at  $k = 6$ ,  $\Delta k = 0.66$  show the same pattern of individuals assigned equally to each cluster in the assignment plots, further suggesting population homogeneity with no substructure (Figure 10). Thus, this result is highly indicative that there is a single, homogenous South African population.

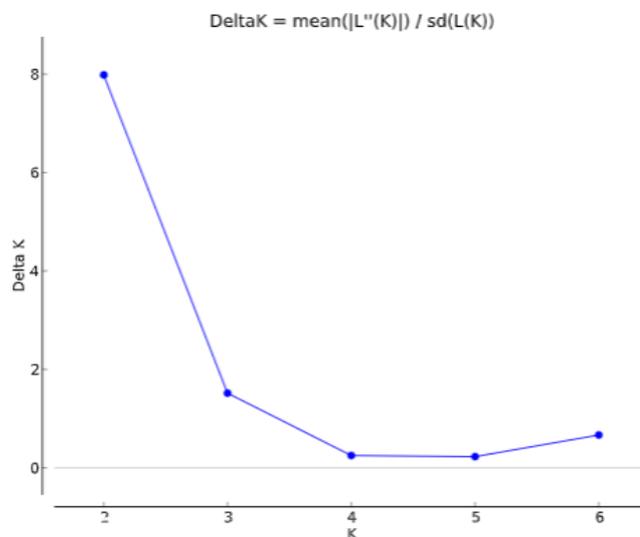


Figure 9. The average DeltaK plotted over the potential number of clusters ( $k$ ). The most likely DeltaK was found to be  $k = 2$ .

We therefore run STRUCTURE a second time for an extended dataset containing additional European samples, we found that the most likely number of admixture proportions was still 2,  $\Delta k = 85.66$ . The South African samples appeared to cluster with one sample from Switzerland in higher proportion (Figure 11), while showing no admixture similarity with any other European country sampled.

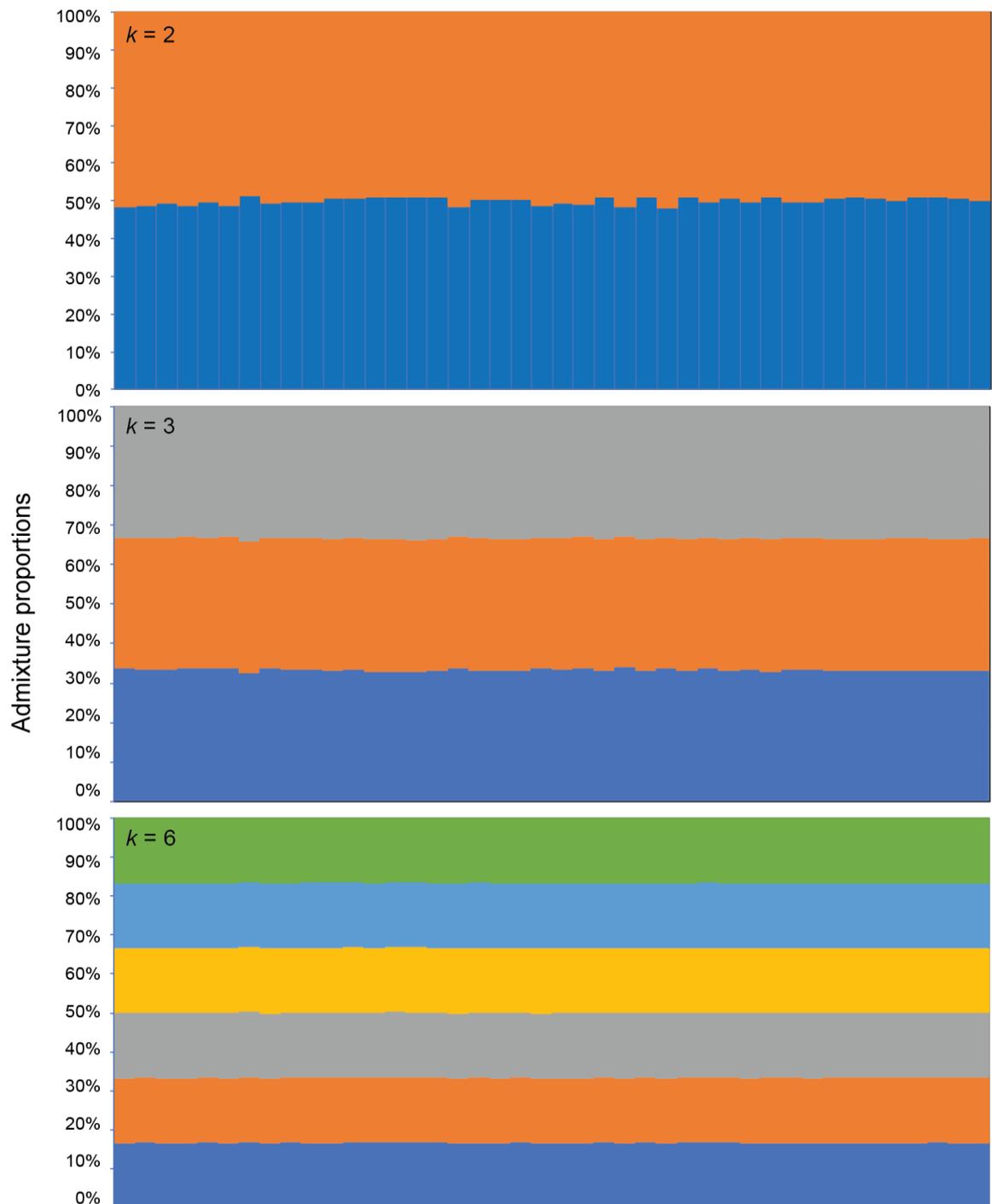


Figure 10. STRUCTURE assignment plots showing the average admixture proportions for *V. germanica* in South Africa. Results inferred based on 9 microsatellite loci. The most likely  $k$  value was  $k = 2$ . Higher levels of  $k$  revealed no further substructure. Secondary optima identified at  $k = 3$  and  $k = 6$  are also shown.

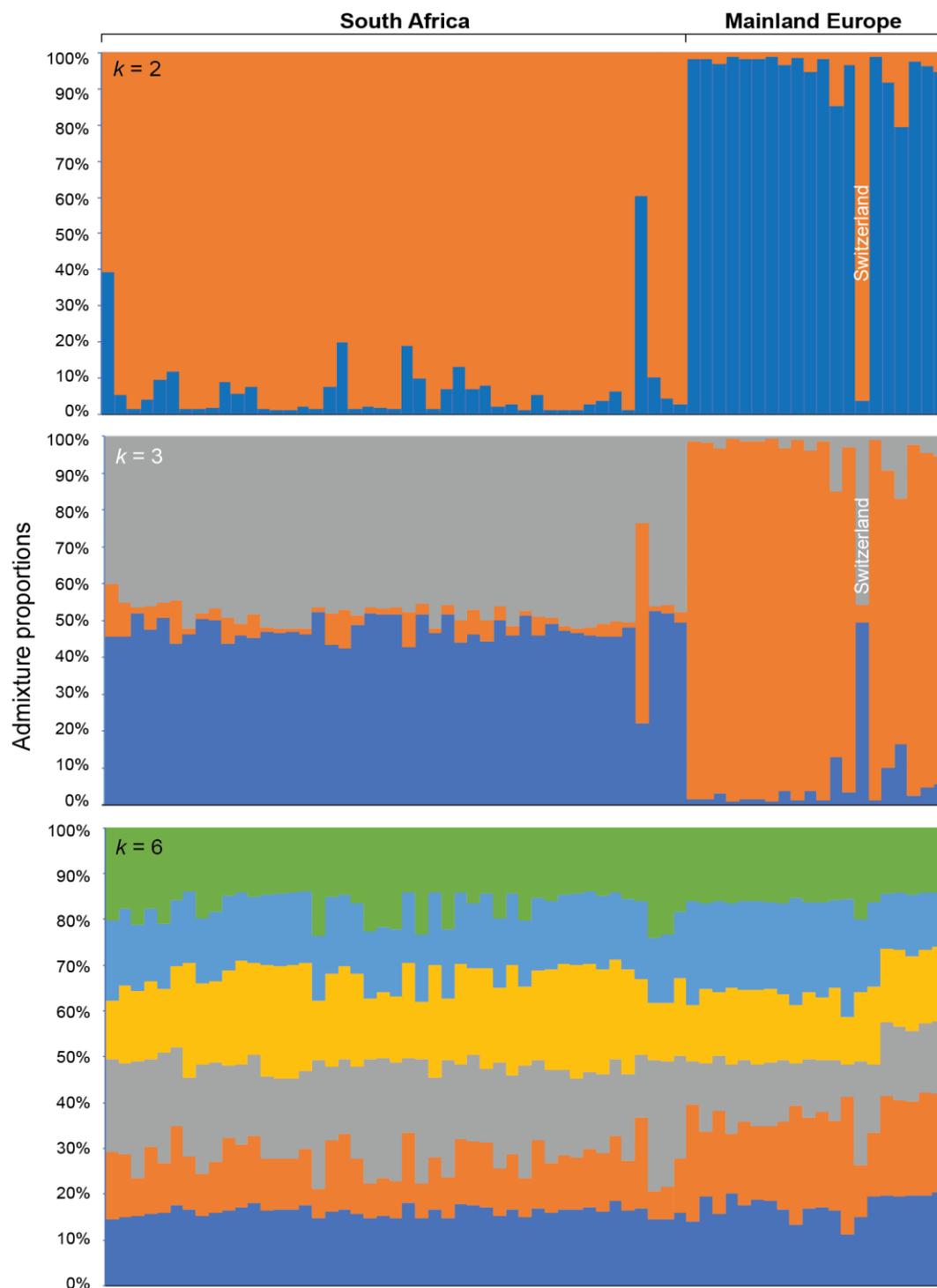


Figure 11. STRUCTURE admixture proportion outputs for *V. germanica* in South Africa and Europe inferred from 7 microsatellite loci. We incorporated data from 45 South African individuals plus 20 European samples. The most likely  $k$  value was  $k = 2$ . Secondary optima identified at  $k = 3$  and  $k = 6$  are also shown.

Due to the lack of substructure in the South African samples, we included 20 European samples from Schmack et al. (2019) in the FCA analyses. The first two axes of the FCA explain 14.8% and 10.8% of the respective variance observed (Figure 12). Two European samples grouped with the South African samples and two of the South African samples plotted within the European samples suggesting shared genetic materials.

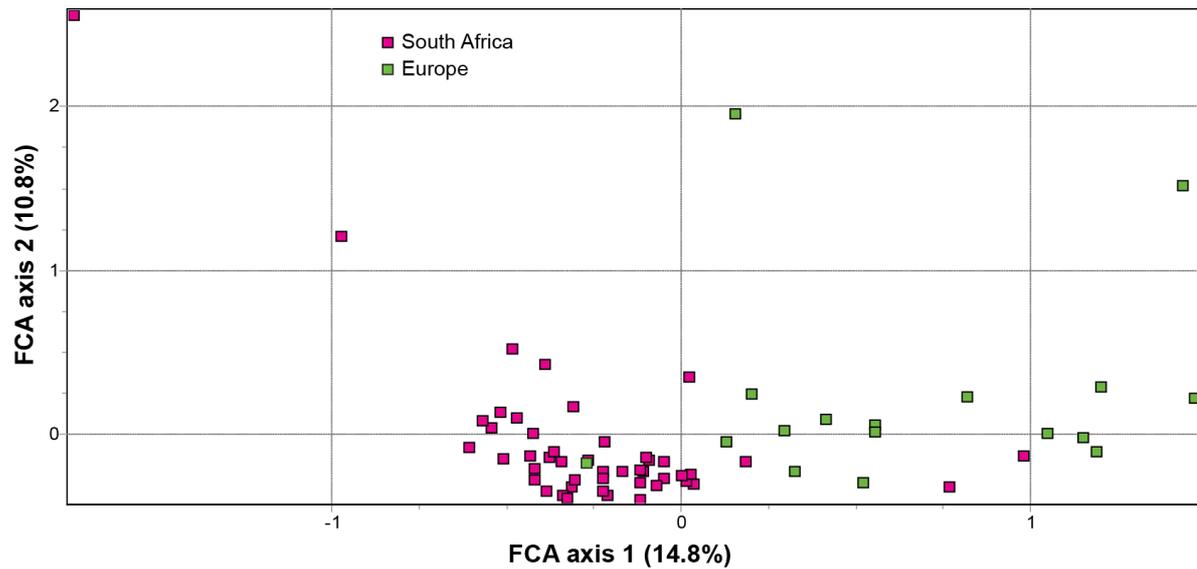


Figure 12. Factorial correspondence analysis of individual multilocus genotypes. Purple squares represent South African wasps and green squares represent European wasps.

## 4. Discussion

### 4.1. Background

Invasive species resulting from human-mediated introductions represent a major threat to biodiversity, agriculture and the economies of invaded regions (Kenis et al. 2009; Pyšek and Richardson 2010; Lester and Beggs 2019). Population genetics analyses are important in biological invasion studies and assists in the design of mitigation strategies to prevent invasive spread (Sakai et al. 2001; Beggs et al. 2011; Masciocchi and Corley 2013; Brenton-Rule et al. 2018). Using genetic markers as a preliminary step minimizes any risks that may be associated with eradication failures, minimizes the costs and environmental impacts associated in eradication processes, and assists in localizing eradication efforts (Abdelkrim et al. 2005). This localization effort is important in the *V. germanica* population of South Africa, as it is still confined to a small area and range expansion has been slow, therefore preventing further spread is of great importance (Veldtman et al. 2012; Haupt 2015; de Villiers et al. 2017; Brenton-Rule et al. 2018). This thesis aimed to address the population genetic structure of *V. germanica* in South Africa, by determining the number of successful establishments, genetic diversity and invasion origins.

### 4.2. Number of successful establishments

In this study, we uncovered six mtDNA haplotypes. Only a single fertilized queen is required for the establishment of a wasp nest and mtDNA is maternally inherited. However, the probability of a single introduced queen to successfully establish a nest and a population is likely to be very small. There needs to be more than one nest producing the next generation of queens and males, otherwise the queens and males produced by that one nest would need to inbreed, resulting in diploid male production. If multiple queens were introduced at the same time, the probability for more than one haplotype being transferred to the new population increases. Therefore, the true number of queen introductions that occurred in South Africa can be inferred to be between two and six (the maximum number of haplotypes). Given that *V. germanica* has been documented in South Africa for approximately 47 years (Veldtman et al. 2012), it is highly unlikely that some of the haplotypes have arisen via recent mutations from the main haplotypes sampled in such short evolutionary time. Previous research outlining the origins of global invasions

suggested that multiple introductions have occurred in South Africa, however the full haplotype diversity was not sampled (Brenton-Rule et al. 2018). Our larger sample size allowed for most mtDNA haplotype diversity to be sampled and confirmed multiple queen introductions.

### **4.3. Genetic diversity of *V. germanica* in South Africa**

Despite the fact that *Vespula germanica* has been documented in South Africa for over 47 years (Whitehead and Prins 1975; de Villiers et al. 2017), their mitochondrial diversity is not surprisingly still low. The phylogenetic tree shows a homogenous population with closely related mtDNA haplotypes. The analyses of the nuclear data also showed no evidence of population sub-structuring within South Africa. This overall lack of genetic diversity could be due to a genetic bottleneck that might have occurred following each introduction with genetic drift acting to decimate variation or high dispersal rates by queens. However, we do not know the levels of genetic variation present in *V. germanica*'s native range; therefore another explanation might be that the species as a whole harbours little genetic variability. We think this latter explanation unlikely due to the extensive geographic distribution of native *V. germanica* populations and the results from a recent study that compared samples of *V. germanica* from Argentina, Australia, New Zealand, and several European countries (Brenton-Rule et al. 2018). The authors found levels of genetic variation in the native range much higher than what we found within South Africa suggesting that bottlenecks might have likely occurred. A genetic bottleneck often occurs following introductions of *Vespula* wasps resulting in a reduction of genetic diversity (Lee 2002). This bottleneck might have occurred initially after the wasps' invasion to South Africa, however, due to the amount of time since the introduction and considering the confined location of the invasion, the lack of variation in this case is most likely due to ongoing gene flow.

There are various factors that could have facilitated the wasp's invasion to South Africa. In some cases invasive success could be due to phenotypical changes and reduced genetic diversity in Hymenopteran invasions (Chapman and Bourke 2001). It has been observed in other social insects such as the Argentine ant, *Linepithema humile*, where despite having low genetic diversity, they have successfully invaded multiple countries worldwide (Ingram and Gordon 2008). The Australian invasion of the Asian honey bee, *Apis cerana*, is an additional example in which despite genetic depletion, a successful establishment still

occurred (Gloag et al. 2007). The haplodiploid sex determination system of *V. germanica* leaves recessive, deleterious alleles exposed to selection (Schmid-Hempel et al. 2007). This exposure to selection in turn results in the offspring of these generations to be more tolerable to an increased level of inbreeding and genetic load. This allows for the successful invasion of *V. germanica* despite the presence of these bottlenecks (Schmid-Hempel et al. 2007; Gloag et al. 2007; Schmack et al. 2019). The cause leading to the lack of genetic diversity, in this case, is therefore most likely due to an initial bottleneck followed by ongoing gene flow. An exchange of genetic material due to queen movement is also considered to influence the invasive success of *Vespula* wasps (Goodisman et al. 2001; Hanna et al. 2014). Invasive *Vespula* wasp colonies possess an increased number of workers produced by multiple queens (Goodisman et al. 2002). Polyandry is also suggested to have helped *Vespula* wasps in other invaded regions (Goodisman et al. 2002; Dobelmann et al. 2017; Schmack et al. 2019), and could also be the case for the South African *V. germanica* population. Further research could be done to determine the level of polyandry in the South African population.

#### **4.4. Origin of invasion**

Previous studies have utilized mitochondrial DNA to determine the origin of invasive wasp populations (Lester et al. 2014; Brenton-Rule et al. 2018). Brenton-Rule et al. (2018) incorporated nine South African samples in their analyses and suggested mainland Europe as the source of the South African introduction. Europe and South Africa have actively been involved in trade relations since the mid-1900's (Taylor 2010). It is therefore no surprise that Brenton-Rule (2018) suggested the source location of the *V. germanica* population is Europe. However, their study lacked any samples from the extensive native range of *V. germanica* outside of Europe. The native range of *V. germanica* is the Palearctic region, its distribution encompasses Europe up to 62°N, the Mediterranean including Algeria and Morocco, and eastwards to northern India, China and Korea to 23°N (Spradbery and Maywald 1992). Therefore, no conclusions can be made on the source of origin of the South African *V. germanica* invasion until extensive comparison with the full native range is conducted. Although our mitochondrial data revealed an Austrian sample as an exact match with the South African wasps we sampled, the second most common mitochondrial haplotype present in South Africa was not detected in the other four European samples we compared them to. The analysis on the combined nuclear dataset grouped the South African samples with a sample from Switzerland. These results could

be suggestive of donor sources to the South African population, however these results are not conclusive until further analysis incorporating samples from throughout the Palearctic region are conducted. This could be an avenue for future research to conduct a more extensive genetic analysis in the native range of *V. germanica* in comparison to their invaded ranges to determine source of origin.

#### **4.5. Applications**

Knowledge of genetic patterns in biological invasions is important in understanding the evolutionary processes leading to the success of an invasion (Sakai et al. 2001). It is also an important step in utilising biological control strategies, as a deeper understanding of the genetic diversity in the invasive region would be required prior to utilising these control agents (Brenton-Rule et al. 2018). Future directions of this research could incorporate the data obtained in this paper to design appropriate invasion control methods suitable to the South African invasive wasp population. A background knowledge of the origin of the invasion could reveal where to find predators or viruses that could be used as a potential biological control agents (Lester et al. 2014). Natural enemies could be searched for in the central-west region of Europe. However, due to the multiple introductions and increased gene-flow, the pathogens that may target *V. germanica* in its native range may not be effective as control agents in the invaded range of South Africa.

Genetically-mediated control is another method of biological control that is becoming of more interest to biologists (Dearden et al. 2018; Brenton-Rule et al. 2018). In invasive populations with high genetic diversity, genetic control methods such as CRISPR/Cas9 drives would not be as useful (Drury et al. 2017). This is due to the fact that the increased diversity may contain genetic variants that promotes immunity toward the gene drive system. The low genetic diversity in the introduced population, such as the South African population, could act as a safety net when utilizing these systems. This is because the probability of the targeted haplotypes to build an immunity toward the system and travel through the population more swiftly are less likely. The FCA analysis also showed a distinction between the South African and European populations despite a few shared genetic materials. This in addition to the geographic separation between these populations reduces the possibility of gene drives making their way back to the native population (Brenton-Rule et al. 2018). In contrast, the lack of subpopulations could result in

recolonization if eradication is localized to one specific area. Thus this form of control would be more suitable on large-scale/area-wide eradication efforts (Esvelt et al. 2014). Due to the presence of multiple introductions in the region, it could mean that future introductions may be likely and therefore eradication would be ineffective as recolonization would occur and disrupt any control attempts. Further efforts should be made to prevent additional introductions prior to eradication efforts. This could be done via increasing biosecurity efforts at the country's borders and main routes of entry.

#### **4.6. Conclusions**

Overall, the South African wasps represent one genetic population with no sub-structuring. Potential causes leading to lack of genetic differentiation may include high dispersal rates as determined for the ant *Formica fusca* (Johansson et al. 2018) and/or genetic drift reducing variability following bottleneck events (Sakai et al. 2001; Azzurro et al. 2006). We also found that the South African German wasp population originated from multiple introductions. These introductions could have occurred independent of one another, or as single events involving multiple queens. It is important that future research try to shed more light on this because if multiple independent introduction events occurred in the past, future introductions may be likely.

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## 6. Supplementary Material

**Supplemental Table 1.** Locality and nest information for *V. germanica* wasps collected for this study and GenBank accession numbers for each of the 3 mtDNA genes. All Individuals were included in the microsatellites analysis but four wasps were excluded from the mtDNA analysis (shown).

Sample name	Country	Locality	Latitude	Longitude	Year collected	Nest #	Accesion # CO1	Accesion # CO2	Accesion # CytB
SA11	South Africa	Kirstenbosch	-33.991605	18.428788	2017	1	MN728034	MN736280	MN736214
SA12	South Africa	Kirstenbosch	-33.991605	18.428788	2017	1	MN728036	MN736299	MN736215
SA13	South Africa	Kirstenbosch	-33.991605	18.428788	2017	1	MN728035	MN736293	MN736217
SA14	South Africa	Kirstenbosch	-33.991605	18.428788	2017	1	MN728040	MN736319	MN736212
SA15	South Africa	South Paarl	-33.781652	19.001263	2017	2	MN728024	MN736328	MN736224
SA16	South Africa	South Paarl	-33.781652	19.001263	2017	2	MN728017	MN736296	MN736266
SA17	South Africa	South Paarl	-33.781652	19.001263	2017	2	MN727992	MN736298	MN736220
SA18	South Africa	South Paarl	-33.781652	19.001263	2017	2	MN728014	MN736324	MN736240
SA19	South Africa	Stellenbosch	-33.948905	18.83713	2017	3	not sequenced	not sequenced	not sequenced
SA20	South Africa	Stellenbosch	-33.948905	18.83713	2017	3	MN728019	MN736307	MN736231
SA21	South Africa	Franschhoek	-33.922598	19.118641	2017	4	MN727998	MN736304	MN736223
SA22	South Africa	Worcester	-33.768784	19.517522	2017	5	MN727984	MN736276	MN736256
SA23	South Africa	Worcester	-33.768784	19.517522	2017	5	MN727983	MN736275	MN736263
SA24	South Africa	Elsenburg	-33.820108	18.840711	2017	6	MN728030	MN736292	MN736246
SA25	South Africa	Ceres	-33.377283	19.305502	2017	7	MN727982	MN736295	MN736233
SA26	South Africa	Wellington	-33.66765	19.042133	2017	8	MN727989	MN736281	MN736257
SA271	South Africa	Devon Valley, Stellenbosch	-33.9490459	18.83825579	2017	9	MN727988	MN736274	MN736265
SA272	South Africa	Devon Valley, Stellenbosch	-33.9490459	18.83825579	2017	9	MN728010	MN736323	MN736232

SA281	South Africa	Babylonstoren, Simondium	-33.8277167	18.9253	2016	10	MN728000	MN736303	MN736225
SA291	South Africa	Babylonstoren, Simondium	-33.8277833	18.92348333	2016	11	MN727995	MN736284	MN736262
SA301	South Africa	Banhoek	-33.9082715	18.94090819	2017	12	MN727993	MN736286	MN736261
SA311	South Africa	Banhoek	-33.9234882	18.93282052	2017	13	MN728009	MN736326	MN736241
SA312	South Africa	Banhoek	-33.9234882	18.93282052	2017	13	MN728005	MN736318	MN736268
SA321	South Africa	Banhoek	-33.9134013	18.94706904	2017	14	MN728025	MN736333	MN736234
SA331	South Africa	Banhoek	-33.9187041	18.95036465	2017	15	MN728022	MN736317	MN736235
SA341	South Africa	Bloukliprivier	-33.9928349	18.87818069	2017	16	MN728041	MN736294	MN736213
SA351	South Africa	Bloukliprivier	-34.004577	18.88727713	2017	17	MN728029	MN736283	MN736250
SA352	South Africa	Bloukliprivier	-34.004577	18.88727713	2017	17	not sequenced	not sequenced	not sequenced
SA361	South Africa	Bloukliprivier	-33.9910522	18.87138443	2018	18	MN728023	MN736285	MN736258
SA362	South Africa	Bloukliprivier	-33.9910522	18.87138443	2018	18	MN727990	MN736330	MN736253
SA371	South Africa	Ceres	-33.3772832	19.30550203	2017	19	MN728002	MN736305	MN736226
SA372	South Africa	Ceres	-33.3772832	19.30550203	2017	19	not sequenced	not sequenced	not sequenced
SA381	South Africa	Ceres	-33.3798941	19.30458175	2017	20	MN728018	MN736325	MN736219
SA391	South Africa	Wynberg, Capetown	-33.9950348	18.41894037	2016	21	MN728037	MN736320	MN736211
SA401	South Africa	Coetzenburg	-33.9468372	18.87190319	2016	22	MN728007	MN736322	MN736251
SA402	South Africa	Coetzenburg	-33.9468372	18.87190319	2016	22	MN728004	MN736279	MN736267
SA411	South Africa	Coetzenburg	-33.9461111	18.87138889	2016	23	MN728003	MN736312	MN736244
SA421	South Africa	Coetzenburg	-33.9450833	18.8696	2016	24	MN728020	MN736301	MN736227
SA431	South Africa	Eerste Rivier	-33.9580827	18.82733125	2017	25	MN728039	MN736316	MN736210
SA441	South Africa	Franschoek	-33.9227389	19.11945522	2017	26	MN728012	MN736315	MN736245
SA442	South Africa	Franschoek	-33.9227389	19.11945522	2017	26	MN727999	MN736309	MN736249
SA451	South Africa	Houtbay	-34.0128843	18.36394151	2016	27	MN728042	MN736334	MN736216
SA461	South Africa	Jonkershoek	-33.9692123	18.93098331	2017	28	MN728026	MN736287	MN736271
SA471	South Africa	Jonkershoek	-33.9604833	18.92275	2016	29	MN728011	MN736329	MN736248

SA481	South Africa	Old Nectar, Jonkershoek	-33.9569184	18.91650218	2018	30	MN728015	MN736288	MN736270
SA482	South Africa	Old Nectar, Jonkershoek	-33.9569184	18.91650218	2018	30	MN727991	MN736273	MN736230
SA491	South Africa	Kleinmond	-34.3354825	19.02626315	2018	31	MN727985	MN736277	MN736272
SA492	South Africa	Kleinmond	-34.3354825	19.02626315	2018	31	MN728021	MN736300	MN736264
SA501	South Africa	Kleinmond	-34.3403558	19.01915594	2018	32	MN728028	MN736313	MN736222
SA511	South Africa	Rivierplaas	-33.7687847	19.51752286	2016	33	MN728013	MN736308	MN736259
SA521	South Africa	Rustenberg Rd	-33.9138775	18.88377099	2016	34	MN728016	MN736311	MN736221
SA522	South Africa	Rustenberg Rd	-33.9138775	18.88377099	2016	34	MN727987	MN736291	MN736228
SA531	South Africa	Rustenberg Rd	-33.89846	18.90232522	2016	35	MN728032	MN736332	MN736236
SA541	South Africa	Somerset	-34.0515285	18.9084314	2016	36	MN727997	MN736321	MN736237
SA551	South Africa	Somerset	-34.0448557	18.91866408	2016	37	MN728008	MN736314	MN736260
SA552	South Africa	Somerset	-34.0448557	18.91866408	2016	37	MN728006	MN736302	MN736242
SA561	South Africa	Stellenbosch	-33.9403203	18.88871389	2016	38	not sequenced	not sequenced	not sequenced
SA571	South Africa	Stellenbosch	-33.9420719	18.87024409	2016	39	MN727994	MN736310	MN736243
SA581	South Africa	Stellenbosch	-33.9429168	18.84960741	2016	40	MN728027	MN736282	MN736247
SA582	South Africa	Stellenbosch	-33.9429168	18.84960741	2016	40	MN727996	MN736306	MN736254
SA591	South Africa	Wellington	-33.6418938	19.02386225	2016	41	MN728001	MN736327	MN736269
SA601	South Africa	Wellington	-33.6416429	19.02380915	2016	42	MN728031	MN736331	MN736229
AU28	Austria	Styria			2012		MN727986	MN736290	MN736255
BL106	Belgium	Leuven			2016		MN728033	MN736289	MN736239
FR26	France	Courtenay			2012		MN727980	MN736278	MN736252
POR1	Portugal	Lisboa, Rua da Mouraria			2012		MN727981	MN736297	MN736218
SP5	Spain	Catalonia, Girona			2012		MN728038	MN736335	MN736238

**Supplemental Table 2.** Showing the DNA concentrations (ng/μl) and quality of the DNA extractions (A20/A280, A260/A2230, A260) recorded for the South African *V. germanica* samples, and the post-dilution concentrations. Bolded values represent extractions that were diluted. (\*) represents a value of low DNA concentration but still amplified in gel electrophoresis.

Sample name	Concentration (ng/μl)	A20/A280	A260/A2230	A260	Concentrations post dilution
SA11	704.15	2.22	2.137	14.73	352.075
SA12	<b>1036.9</b>	2.233	2.127	22	518.45
SA13	498	2.118	2.265	10.44	498
SA14	505.35	2.199	2.216	14.7	252.675
SA15	598.2	2.095	1.304	14.38	299.1
SA16	242.05	2.144	1.733	4.966	242.05
SA17	697.3	2.219	1.75	15.07	348.65
SA18	317.2	2.145	1.603	7.023	317.2
SA19	345.8	1.977	1.245	9.568	345.8
SA20	<b>*54.9</b>	1.041	0.56	2.08	54.9
SA21	<b>1017.8</b>	2.291	2.065	22.43	508.9
SA22	<b>1045.1</b>	2.264	1.939	22.52	522.55
SA23	<b>1166</b>	2.273	2.159	27.03	583
SA24	787.2	2.198	1.823	18.44	393.6
SA25	229.4	1.942	1.296	6.327	229.4
SA26	634	2.173	1.851	14.52	317
SAB271	598.85	2.194	1.867	12.86	598.85
SAB281	599.55	2.186	1.867	12.86	599.55
SAB291	772.55	2.197	1.908	16.27	386.275
SAB301	863.55	2.209	1.938	17.94	431.775
SAB311	804.2	2.124	1.812	18.25	402.1

SAB321	<b>1165.9</b>	2.275	2.111	23.97	582.95
SAB331	769.25	2.193	1.929	16.15	384.625
SAB341	<b>1044.7</b>	2.246	1.956	2171	522.35
SAB351	669.35	2.173	1.722	14.33	334.675
SAB361	975.8	2.216	1.813	20.78	487.9
SAB371	<b>1166.8</b>	2.221	1.685	24.79	583.4
SAB381	803.85	2.191	1.983	16.74	401.925
SAB391	893.25	2.215	2.155	18.4	446.625
SAB401	<b>1202.5</b>	1.299	1.247	25.06	601.25
SAB411	765.4	2.252	1.843	20.54	382.7
SAB421	<b>1094.5</b>	2.297	2.146	22.54	547.25
SAB431	675.5	2.221	1.961	14.7	337.75
SAB441	<b>1128.3</b>	2.292	2.032	23.35	564.15
SAB451	587.05	2.192	2.056	13.88	587.05
SAB461	660.75	2.215	2.23	13.34	330.375
SAB471	<b>1092.2</b>	2.168	1.95	22.78	546.1
SAB481	617	2.217	1.958	13.74	308.5
SAB491	621.1	2.205	2.203	12.54	310.55
SAB501	419.8	2.19	2.185	8.451	419.8
SAB511	823.2	2.195	1.986	18.07	411.6
SAB521	298.55	2.156	1.969	6.142	298.55
SAB531	699.85	2.233	1.986	14.63	349.925
SAB541	<b>914.1</b>	2.206	2.076	18.91	457.05
SAB551	467.55	2.181	1.749	12.12	467.55
SAB561	<b>1113.6</b>	2.296	2.028	22.94	556.8
SAB571	<b>935.1</b>	2.26	2.062	19.38	467.55
SAB581	443.2	2.178	1.856	9.281	443.2
SAB591	823.8	2.215	1.903	17.34	411.9

<b>SAB601</b>	616.4		2.199	2.053	12.87	308.2
<b>SAB272</b>	488.8		2.192	2.159	10.08	488.8
<b>SAB312</b>	596.9		2.255	2.225	12.24	596.9
<b>SAB352</b>	600.25		2.219	1.886	12.67	300.125
<b>SAB362</b>	580.4		2.219	2.086	12.01	580.4
<b>SAB372</b>	543.1		2.215	1.878	11.69	543.1
<b>SAB402</b>	630.45		2.221	2.093	13.15	315.225
<b>SAB442</b>	<b>1253.1</b>		2.327	2.029	26.55	626.55
<b>SAB482</b>	562.9		2.24	2.023	11.62	562.9
<b>SAB492</b>	798.6		2.223	2.144	16.46	399.3
<b>SAB522</b>	427.9		2.186	1.874	9.03	427.9
<b>SAB552</b>	759.1		2.22	1.849	16.27	379.55
<b>SAB582</b>	764.45		2.249	1.969	16.11	382.225

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**Supplemental Table 3.** Microsatellite genotypes for *V. germanica* screened in this study.

Sample ID	LIST2007		VMA-6		VMA-7		Rufa 5		Rufa19		R4-114		LIST2011		LIST2019		LIST2003		List2004	
SA11	180	180	302	302	157	157	161	163	222	224	133	135	143	149	161	161	238	244	177	180
SA12	180	180	302	302	157	157	161	161	212	220	133	135	143	149	161	161	241	244	177	180
SA13	180	180	302	302	157	157	159	161	220	224	133	135	143	143	161	161	238	241	177	180
SA14	180	180	302	302	157	157	161	161	222	222	133	135	143	149	161	161	238	241	177	180
SA15	180	182	300	304	157	157	167	167	212	220	133	135	149	149	161	161	238	238	174	180
SA16	180	182	302	302	157	157	161	167	212	218	133	133	149	149	161	161	241	241	177	180
SA17	180	182	300	302	157	157	163	167	220	220	133	133	149	149	161	161	238	241	177	180
SA18	180	182	300	302	157	157	163	167	220	220	133	135	149	149	161	161	238	238	177	180
SA19	180	180	298	302	157	157	161	167	218	218	133	135	143	149	161	161	241	241	177	180
SA20	180	180	298	302	157	157	161	167	218	218	133	135	143	149	161	161	241	244	180	183
SA21	180	180	298	300	157	157	161	161	212	218	133	135	143	149	161	161	238	244	174	183
SA22	180	180	300	300	157	157	161	161	218	220	135	135	143	149	161	161	241	241	177	180
SA23	180	180	300	300	157	157	161	167	212	218	135	135	143	149	161	161	241	241	173	180
SA24	180	180	298	300	157	157	161	161	218	220	133	135	149	149	161	161	238	244	174	180
SA25	180	182	302	302	157	157	161	163	218	218	133	133	149	149	161	161	238	241	180	183
SA26	180	180	300	302	157	157	167	167	212	220	133	135	149	149	161	161	241	244	180	183
SAB271	180	180	300	302	157	157	163	163	212	218	133	135	149	149	161	161	241	244	177	180
SAB281	180	182	308	308	157	157	161	163	212	218	133	135	149	149	161	161	235	238	174	183
SAB291	180	180	302	308	157	157	159	161	218	218	133	133	149	149	161	161	238	241	180	183
SAB301	180	180	300	300	157	157	161	161	212	218	135	135	149	149	161	161	241	241	180	183
SAB311	180	182	300	302	157	157	161	167	218	218	133	133	149	149	161	161	241	241	180	180
SAB321	180	182	302	302	157	157	163	167	212	218	133	133	149	149	161	161	238	241	180	183
SAB331	180	180	302	302	157	157	163	163	212	218	133	133	149	149	161	161	241	241	180	183
SAB341	180	180	300	302	157	157	161	163	218	218	133	135	149	149	161	161	241	244	180	183
SAB351	180	180	300	302	157	157	161	167	212	212	133	135	143	159	161	161	238	238	177	180
SAB361	182	182	302	308	157	157	161	163	212	220	133	135	149	149	161	161	238	238	180	183
SAB371	180	182	302	302	157	157	159	161	218	218	133	133	149	149	161	161	238	241	174	180
SAB381	180	182	302	302	157	157	161	163	218	220	133	133	143	149	161	161	238	241	180	183
SAB391	180	180	302	302	157	157	161	163	212	220	133	135	143	149	161	161	244	244	177	180
SAB401	180	180	300	302	157	157	161	161	212	220	133	133	149	149	161	161	238	238	177	180
SAB411	180	180	300	302	157	157	163	167	212	212	133	135	149	149	161	161	238	244	177	180
SAB421	180	182	300	302	157	157	161	161	218	218	135	135	149	149	161	161	238	238	180	183
SAB431	180	180	300	302	157	157	161	167	212	220	135	135	149	149	161	165	241	244	177	180
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SAB451	180	180	298	302	157	157	161	163	220	222	133	133	143	149	161	161	244	244	177	180
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SAB272	180	180	300	300	157	157	163	163	212	218	133	133	149	149	161	161	238	244	180	183

SAB312	180	180	300	302	157	157	161	167	218	218	133	133	149	149	161	161	241	244	180	183
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SAB372	180	182	300	302	157	157	163	167	218	218	133	133	143	149	161	161	238	238	177	180
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SAB492	182	182	300	302	157	157	161	161	218	218	133	135	149	149	161	161	238	238	180	183
SAB522	180	180	300	300	157	157	161	163	220	220	133	133	149	149	161	161	241	241	177	180
SAB552	180	182	302	308	157	157	160	162	216	218	133	135	143	149	161	161	235	244	180	183
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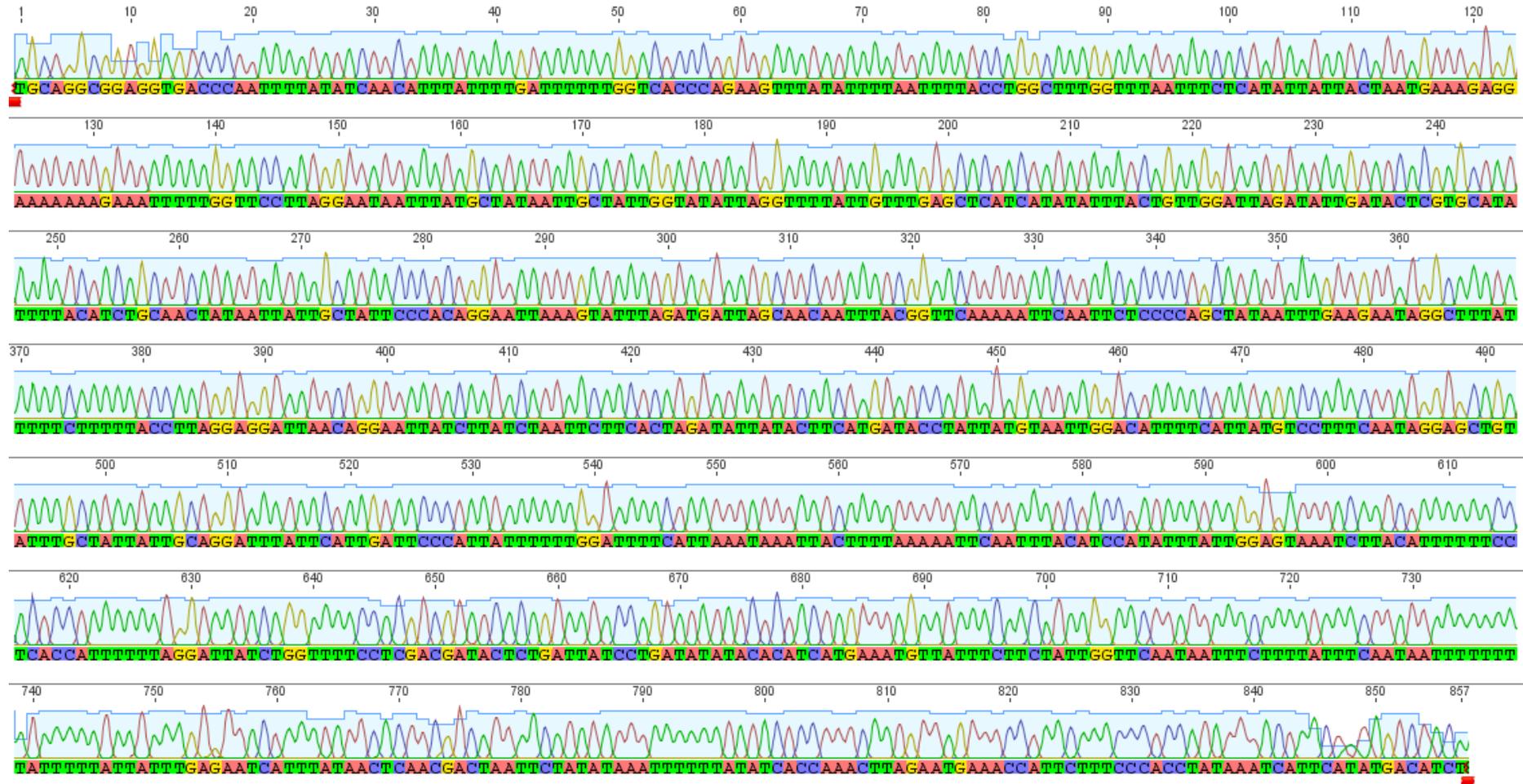
## Supplemental Figures

**Supplemental Figure 1.** Electropherogram example (CO1) from this study. The numbers above the electropherogram represent the bp position. The blue bars in the background represents the quality of the sequence. Low quality ends were trimmed to ensure only high quality base calling was used in the study. All sequences have been uploaded to GenBank with accession numbers: MN727980-MN728042, MN736210-MN736272 and MN736273-MN736335.

**Supplemental Figure 2.** Truncated aligned sequences as they appear in Geneious, indicating the polymorphic regions and positions where SNP's occur.

**Supplemental Figure 3.** Maximum composite likelihood tree for *V. germanica* throughout the South African range ( $n = 58$  wasps), and mainland Europe ( $n = 5$ ). The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985). The tree with the highest log likelihood (-1966.2129) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 63 nucleotide sequences, incorporating 5 individuals from mainland Europe, and 58 individuals from 41 nests across the South African invaded range. Individuals with a 2 following the name represent an additional individual sampled from a nest. There were a total of 1518 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Tamura et al. 2011).

**Supplemental Figure 4.** Electropherogram example of a microsatellite run on the ABI sequencer for mix 2. Each of the Loci were tagged with an M13-primer labelled with one of four dyes; 6-FAM (blue), HEX (green), PET (red), NED (yellow). The GeneScan 500 ladder is represented by the orange peaks.



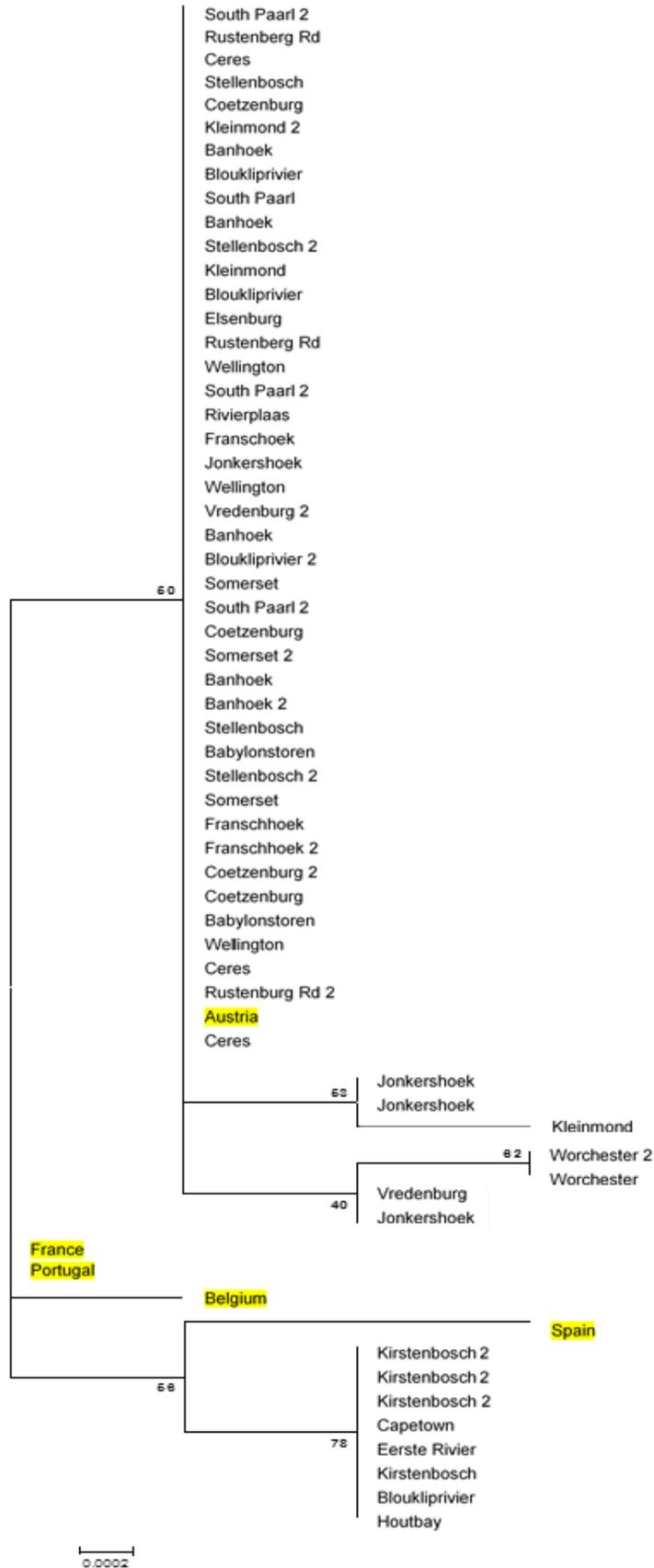
Supplemental figure 1



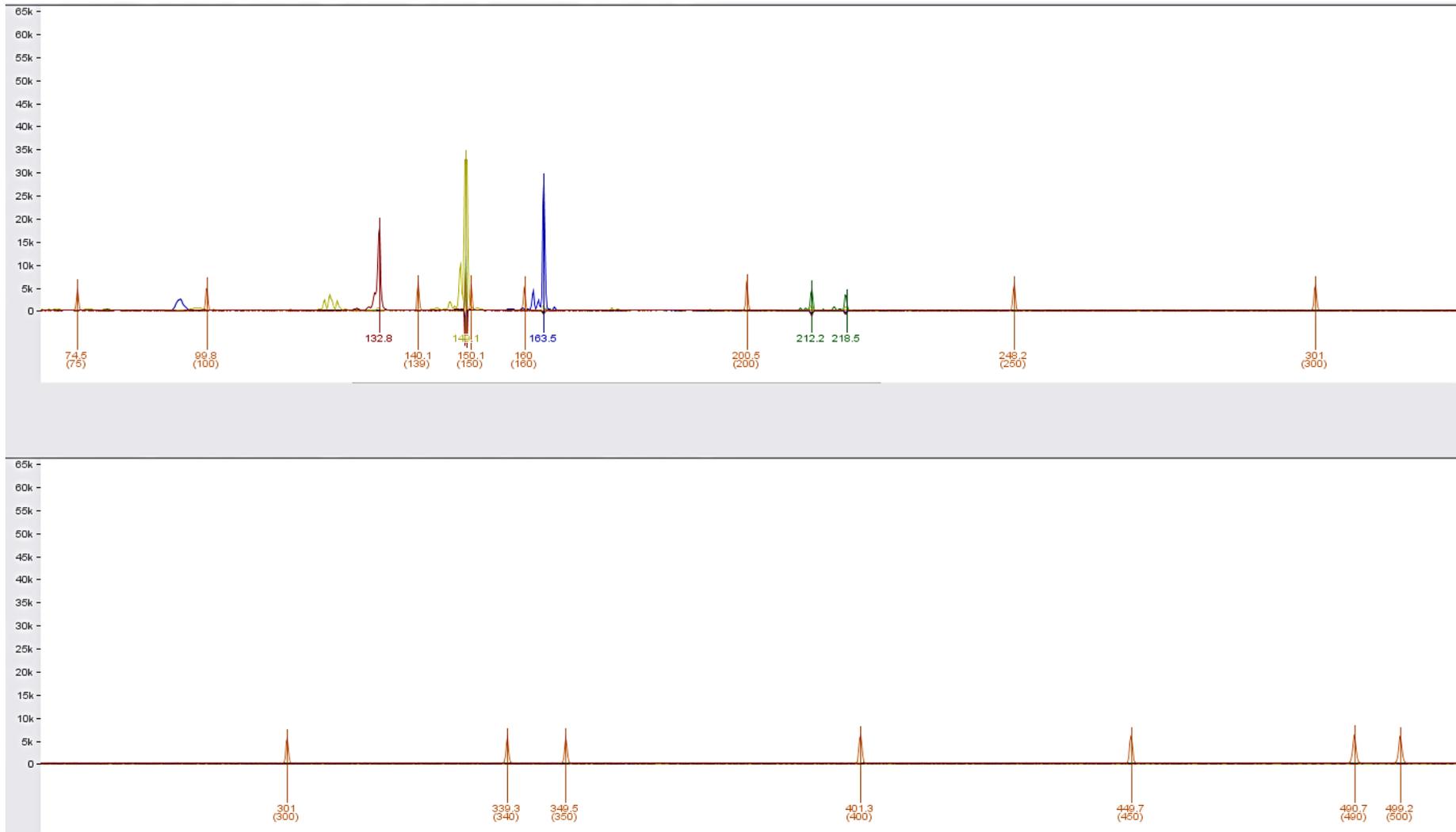








Supplemental figure 6



Supplemental figure 7