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Running header: Origin of & enemy release in an invasive wasp

Determining the origin of invasions and demonstrating a lack of enemy release from microsporidian pathogens in the common wasps (*Vespula vulgaris*)

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ABSTRACT

Aim Understanding the role of enemy release in biological invasions requires an assessment of the invader's home range, the number of invasion events, and enemy prevalence. The common wasp (*Vespula vulgaris*) is a widespread invader. We sought to determine the Eurasian origin of this wasp and examined worldwide populations for microsporidian pathogen infections to investigate enemy release.

Location Argentina, Eurasia, New Zealand

Methods A haplotype network and phylogenetic tree were constructed from combined wasp *COI* and *cytb* mitochondrial markers. A morphometric study using canonical discriminant analysis was conducted on wing venation patterns. Microsporidian pathogens prevalence was also examined using small subunit rRNA microsporidia-specific primers.

Results Our spatially structured haplotype network from the native range suggested a longitudinal cline of wasp haplotypes along an east to west gradient. Six haplotypes were detected from New Zealand, and two from Argentina. The populations from the introduced range were genetically similar to the western European, UK and Ireland. The morphometric analysis showed significant morphological variation between countries and supported the Western European origin for New Zealand populations, though not for Argentine samples. Microsporidian infection rates were highest in New Zealand samples (54%), but no significant differences in infection rates were observed between the invaded and native range. *Nosema* species included matches to *N. apis* (a pathogen from honey bees) and *N. bombi* (from bumble bees).

Main conclusions Multiple introductions of the common wasp have occurred in the invaded range. A high microsporidian infection rate within the native range, combined with multiple introductions and a reservoir of pathogens in other social insects such as bees, likely

contributes to the high microsporidian infection rates in the invaded range. Enemy release is likely to be more frequent when pathogens are rare in the home range, or are host-specific and rare in reservoir populations of the introduced range.

Keywords

Biological invasion, enemy release, *Nosema*, pathogen, social wasp, *Vespula vulgaris*

INTRODUCTION

The enemy release hypothesis proposes that invasive species can become abundant because of the absence of co-evolved natural enemies such as pathogens and parasites (Keane & Crawley 2002; Torchin *et al.* 2003). Reduced enemy abundances are, however, likely to occur only under certain circumstances. The more often an invader is introduced, the more likely natural enemies will also be introduced. The probability of the introduction of enemies must also be related to their prevalence in host populations within the native range, as a high pathogen infection rate of an invasive species within their native range must correspond to a high probability of any invasive propagules being infected. The host specificity of natural enemies likewise must influence the prevalence and effects of natural enemies on invaders. Should existing exotic or native natural reservoirs of natural enemies occur in a new environment, they may spillback to arriving invasive species (Flory & Keith, 2013). The potential role of alternative hosts for pathogens in maintaining, or in some situations reducing, the presence and abundance of pathogens in an environment has been clearly demonstrated with Lyme disease (Ostfeld & Keesing, 2000).

The substantial influence that natural enemies such as pathogens can have on populations of social insects is exemplified in the global decline of bee populations. Colony

collapse disorder is associated with widespread loss of honey bees. The exact causes of this disorder are currently unknown, but likely involve a combination of several pathogens or parasites and perhaps other factors such as pesticide exposure (Bromenshenk *et al.*, 2010; Evans & Schwarz, 2011). Bumble bees are also experiencing a substantial population decline associated with pathogens and low genetic diversity (Cameron *et al.*, 2011). Pathogens thought to be responsible for bumble bee and honey bee declines include microsporidians in the genus *Nosema*. These studies suggest that pathogens can have a major effect on social insects, which may be compounded by factors such as low genetic diversity. Invasive social insects typically have low mean population genetic diversity as a result of a limited number of invasive propagules (e.g. Corin *et al.*, 2007; Gruber *et al.*, 2012).

The common wasp (*Vespula vulgaris*) is an invasive species of major biodiversity and conservation importance. Their high densities in the invaded range of countries such as New Zealand and Argentina are the driver of their substantial ecological impacts. In New Zealand, for example, densities of up to 370 wasps m^{-2} of tree trunk (Moller *et al.*, 1991) and 34 nests ha^{-1} (Beggs *et al.*, 1998) have been observed. Under such conditions the probability of an orb-web spider surviving to the end of a wasp season has been estimated at virtually zero (Toft & Rees, 1998). Common wasps also compete with native birds and have been recorded attacking and killing chicks (Moller, 1990). These wasps are native to and widespread in Eurasia (Archer, 1989; Dvořák, 2007). Within this native range, population densities appear to fluctuate dramatically. Exceptional years of high abundance in the native range are frequently followed by years of scarcity, with queen productivity varying by a factor of 100 between nests and years (Archer, 1981). These results indicate some form of endogenous density-dependence and additional exogenous factors such as climate may promote variation in abundance (Archer, 1981; Archer, 1985). Alternatively, fluctuations in wasp abundance may be driven by pathogens and parasites. Such natural enemies are prevalent in *Vespula* spp.

wasp populations within their native range (Rose *et al.*, 1999; Evison *et al.*, 2012), though their abundance and influence in the introduced range is unknown.

A critical first step towards identifying co-evolved pathogens and assessing whether enemy release has occurred is to identify the home range. In the absence of the knowledge of a specific home range, the degree of enemy release may be overestimated (Colautti *et al.*, 2005). The matching of an invasive species to their specific origin can be essential for the successful selection of a haplotype or genotype for biological control (Goolsby *et al.*, 2006). Molecular methods have been particularly useful for determining the home range of invasive plants and animals, indicating invasion pathways and origins for a range of species (e.g. Goolsby *et al.*, 2006; Corin *et al.*, 2007). Morphological variation may also be useful for identifying intraspecific genetic variation and areas of origin (Nielsen *et al.*, 1999; Abramoff *et al.*, 2004; Al-Ghamdi *et al.*, 2013). Our first aim in this study was to estimate the specific area within the Eurasian host range from which the New Zealand and Argentinian populations of the common wasp originated, using variation in mitochondrial DNA and morphology within the home range. We examined wasps from throughout the invaded range in New Zealand and Argentina to search for evidence of multiple successful introductions. Our second aim was to assess whether enemy release occurs in common wasp populations outside the home range by comparing the prevalence of *Nosema* microsporidian pathogens in the invaded and native range. Microsporidian pathogens may be important for in bee colony collapse disorder (Bromenshenk *et al.*, 2010; Evans & Schwarz, 2011). Moreover, these pathogens have also been demonstrated to infect and multiply in *Vespula* wasps, with the potential to kill entire wasp nests (Fantham & Porter, 1913).

METHODS

Samples

Wasps were obtained by contacting entomologists located throughout the native and invaded range. Samples of foraging workers or workers from nests were either freshly collected for this study, or were from preserved samples (Fig. 1; Supplementary material Table S1). If wasps were collected from nests, only a single individual from the nest was subsequently used in the analysis. We note that while we achieved collection of samples from a representative distribution of these wasps, given their wide distribution it is likely that we have missed genetic diversity occurring in under-sampled regions such as Asia and Russia. Common wasps were only recently detected in Argentina (Masciocchi *et al.*, 2010) and the samples from this country spanned their entire known distribution at the time of collection (February and March 2013). Australia has been invaded by the common wasp (Richards, 1978), but despite attempts to collect fresh samples no Australian specimens were obtained and only two historic samples from the 1970s were sourced. DNA extractions from these specimens were not successful. Australian entomologists we contacted suggested that common wasp populations had been superseded by the more recently arrived German wasp (*V. germanica*). For the phylogenetic analysis, we used an individual German wasp (*V. germanica*) as an outgroup, which was collected in Auckland, New Zealand. Note that the range of the common wasp was previously thought to extend into North America, although recent work has demonstrated '*V. vulgaris*' in this region are actually *V. alascensis* (Carpenter & Glare, 2010).

Wasp phylogenetic relationships

To ascertain the phylogenetic relationships and prevalence of microsporidian infections among the *V. vulgaris* samples from the native and introduced ranges, we sequenced DNA

from wasp workers sampled throughout these ranges (Fig. 1). Wasps were dissected and the gut used for both wasp DNA and microsporidian extractions (these are primarily gut parasites). We extracted DNA using a standard digestion with 0.2% SDS and 0.5 mg/ml proteinase-k until the tissue dissolved (2-4 h), followed by phenol/chloroform purification, ethanol precipitation and re-suspension in Tris-EDTA buffer.

We used PCR to amplify portions of the mitochondrial loci *COI* (cytochrome oxidase I) and *cytb* (cytochrome b) for phylogenetic analysis. The mitochondrial primers were C1-J-1718(Sid) 5'-GGA GGA TTT GGA AAT TGG CTT ATT CC-3' and C1-N-2191(Nancy) 5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3' for *COI*, and CB1 5'- TAT GTA CTA CCA TGA GGA CAA ATA TC-3' and CB2 5'- ATT ACA CCT CCT AAT TTA TTA GGA AT-3' for *cytb* (Simon *et al.*, 1994). Each 15 µl PCR reaction consisted of 1 µl of template DNA (~20 ng DNA), 1 X PCR Buffer, 0.4 mg/mL of bovine serum albumin (BSA), 1.5 mM MgCl2, 200 µM of each dNTP, 0.4 µM of each primer and 0.1 Unit of Taq DNA Polymerase (Fisher). Thermal cycling conditions for *COI* and *cytb* consisted of initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturing at 94°C for 30 s, annealing for 40 s at 45°C, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. Amplified products were purified using ExoSAP-IT (US Biochemicals, Cleveland, Ohio) and sequenced directly with an ABI 3730XL Genetic Analyser (Applied Biosystems) by Macrogen Inc., Seoul, Korea and the Massey Genome Service, Palmerston North, New Zealand. Genomic DNA was sequenced from 103 samples. We manually checked for quality, edited and aligned the DNA sequences using MEGA5.1 (Tamura *et al.*, 2011). The sequence of the sample from Mongolia was of poor quality and was discarded from further analysis. We used BLASTn searches of the NCBI (GenBank) nucleotide (nr) database to confirm the authenticity of our 105 clean sequences as *V. vulgaris* (and the outgroup *V. germanica*). For

our phylogenetic analysis we assessed the *cytb* (420 bases) and *COI* datasets (432 bases) separately, and both sets of sequences as a concatenated dataset (852 bases).

To determine the most appropriate model of sequence evolution for our datasets, we used Bayesian Information Criterion (BIC) scores derived in MEGA, which also estimated base frequencies, substitution rates, the proportion of invariable sites (I), and the uniformity of substitution rates among sites (G). The models of evolution selected as best-fitting differed slightly for the three datasets, but the best-fitting model for the concatenated dataset also ranked among the three best models for the cytb and *COI* datasets using BIC scores (Supplementary material Table S2). In addition, the Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985) with a gamma distribution parameter (HKY + G model) ranked among the four best-fitting models for all three datasets using Akaike Information Criterion (AICc) and Maximum Likelihood (lnL) values. We therefore considered the models of evolution to be comparable and used the model selected for the concatenated dataset (HKY+G=0.16) for tree-building. The estimated model and parameters were then used to generate a Maximum Composite Likelihood (MCL) tree and the level of support was assessed with 2000 bootstrap replicates in MEGA. We also used MEGA to calculate percentage genetic distances and standard errors (S.E.) among groups of individuals. The HKY model is not implemented in MEGA for genetic distance calculation, so we used the Tamura-Nei model (TN93; Tamura & Nei, 1993) with a gamma parameter of 0.16 as this was among the best-fitting models for the concatenated dataset.

We visualised the relationships among mitochondrial haplotypes and regions by creating a spatially structured haplotype network in TempNet (Prost & Anderson, 2011). We grouped samples into seven geographical regions: Asia (China, Mongolia); Eastern Europe (Poland, Hungary, Austria, Czech Republic, Greece, Slovakia); Northern Europe (Russia,

Estonia, Finland, Sweden); Western Europe (France, Belgium, Germany, Italy, Spain); UK & Ireland (England, Ireland, Northern Ireland).

To test the closest genetic relationships of samples from individual regions to our New Zealand and Argentine samples, we used generalised linear models (GLM) with a negative binomial distribution and log link function using the *MASS* package (Venables & Ripley, 2002) in R version 2.15.1 (Ihaka & Gentleman, 1996; R Development Core Team, 2012). Genetic distance to the New Zealand or Argentinian samples was modelled as the response variable and region as the predictor variable. To assess correlations between geographic and genetic distance (i.e. isolation by distance) we conducted a Mantel test using the *ade4* package (Dray & Dufour, 2007) in R, using 9999 replicates.

Wasp wing morphology

In addition to genetic markers we examined variation in wing morphology as another potential character that may be useful to derive the area of origin for the invasive populations. Wing morphology has been utilized to successfully derive sub-species status in other hymenopteran populations, such as honey bees (e.g. Ruttner *et al.*, 1978). The right forewing of adult worker wasps was removed and placed on a microscope slide, with an additional slide placed on top to flatten the wing. A digital photograph was taken of the wing and the distances between 25 wing nodes were measured using ImageJ (Abramoff *et al.*, 2004; Al-Ghamdi *et al.*, 2013). To account for variation in wasp size, data were standardized by dividing the distance between two nodes by the distance between the nodes b and i (Fig. 2a). We only analysed samples from countries from which we had ≥ 10 individual wasps.

To examine variation in wing morphology between wasp populations in the native and invaded range of *V. vulgaris*, we used canonical discriminant analysis in the *candisc* package (Friendly & Fox, 2013) in R. We first utilized stepwise discriminant function analysis to

reduce the number of variables in an attempt to avoid issues associated with co-linearity. Country was used as the discriminator variable and "unexplained variance" used as the stepwise method. The F-value to enter variables was set at $\alpha < 0.05$, and minimum significance to remove variables at $\alpha > 0.10$. The *candisc* output includes a Type II MANOVA, which tested the hypothesis that there is morphological differentiation amongst the populations.

Microsporidian pathogen prevalence

Small sub-unit rRNA (SSU rRNA) general microsporidia-specific primers, which amplify the V1-V3 regions of the 16S (SSU) gene were used for the *Nosema* assay. The primers were V1f 5'-CAC CAG GTT GAT TCT GCC TGA C-3' and 530r 5'-CCG CGG CTG CTG GCA C-3' (Baker *et al.*, 1995) used the same DNA extractions as for the phylogenetic analysis, together with additional extractions, and analysed all samples for microsporidian infection. Thermal cycling conditions included initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturing at 95°C for 40 s, annealing at 60°C for 40 s, and extension at 72°C for 40 s, followed by a final extension at 72°C for 10 min. Triplicates of the microsporidian pathogen assays were run to estimate false negative rates using the equation $1-(a/(b*3))$, where a was the total number of positive amplifications and b was the number of samples that resulted in at least one positive amplification. Amplified products of microsporidian-positive PCR products were purified and sequenced as for the wasp samples (n = 51). Although the resulting sequences (186 bases) were of sufficient resolution to enable identification to genus level (and species in some cases), low electropherogram peaks prevented a robust examination of phylogenetic similarity or sequence diversity. We used BLASTn searches of the NCBI (GenBank) nucleotide (nr) database to identify the closest matching species to our sequences.

Estimates of mean microsporidian pathogen infection rates (± 95% confidence intervals) were calculated using 10,000 bootstraps in IBM SPSS v 20.0 (IBM Corp, 2011). Infection rates were considered significantly different if confidence intervals did not overlap.

RESULTS

Wasp phylogenetic relationships

Our phylogenetic analysis revealed 33 unique *V. vulgaris* haplotypes among our samples (Fig. 3). The New Zealand samples were closest in genetic distance to those from Argentina, the United Kingdom, France and Belgium (Fig. 1, Table S4). Six haplotypes were detected among the New Zealand samples (Fig. 4), which grouped closely with Argentine haplotypes (Fig. 3 and Fig. 4). Our spatially structured haplotype network suggested a longitudinal cline of haplotype diversity in the native range, with the eastern European populations more similar to the Asian populations, and UK & Ireland populations more similar to western European populations. The populations from the invaded range were more similar to the western European, UK and Ireland haplotypes (Fig. 4 & Fig. S1A).

The genetic distance between the New Zealand samples and all other regions except Argentina was significantly greater than within New Zealand samples (Fig. S1, Table S3). The genetic distance between the Argentine samples and other regions, compared to the genetic distance between the Argentine samples followed a similar pattern to that of New Zealand. Again, differences were not significant between Argentina and New Zealand (Fig. S1, Table S3). The results of these tests were consistent with the spatial structure observed in haplotype diversity and genetic distance, with the closest relationships between the native range and New Zealand and Argentinian samples being those from the UK and Ireland. Our Mantel test found a significant correlation between genetic and geographical distance in the

native range (Fig. S4, r=0.838, p<0.001) consistent with a longitudinal cline in genetic variation.

Wasp wing morphology

We analysed for morphological differences between the wasp populations from different countries or regions. Using these standardized measurements, the canonical discriminant analysis indicated significant population differentiation (MANOVA $F_{9,117} = 3.827$, $P < 0.001$). The stepwise function of the analysis used 13 of the 25 possible measurements (Fig. 2b: relative distances between a-b, c-d, d-e, e-f, j-k, k-l, n-o, q-r, b-p, e-q, h-r). New Zealand samples grouped near the UK, Ireland, Belgium and France (Fig. 2b). Argentinian samples were the most differentiated based on the canonical axis 1, which explained 43.6% of the morphological variation. The measurement giving the highest proportionate differentiation between the New Zealand and Argentinian samples was e-q (Fig. 2a), which indicated this measurement was on average 9.3% smaller in Argentine samples relative to New Zealand specimens.

Microsporidian pathogen prevalence

Our microsporidian pathogen assays revealed that the closest matching sequences on GenBank were typically *Nosema* spp. with percentage cover of 97 - 100% and identity of 97 - 100% in the majority of cases (n = 57/67). Some sample sequences (n = 6) were less well-resolved (70-97% cover and 79-92% identity), and four sequences were unresolved. Specific *Nosema* species matches on GenBank were to *N. apis* (two samples from Belgium and two samples from Slovakia with 100% cover and 100% identity), and *N. bombi* (four samples from New Zealand with 100% cover and 98% identity). Nucleotide signals in the

electropherograms for a number of sample sequences were weak. Given these weak signals we were not confident in undertaking any further analysis of microsporidian diversity.

The false negative rate among all samples was 56.86%, which may account for the higher microsporidian infection observed here relative to studies such as Evison *et al.* (2012). The prevalence of microsporidian pathogen infection among regions ranged from 0% (Asia, n = 2) to 54% (New Zealand, n = 39; Table 1), however, the variation in infection rates among regions was not significant given that the 95% confidence intervals for the infection rates of all regions overlapped (Table 1).

DISCUSSION

Understanding the role of enemy release in biological invasions requires an accurate assessment of the invader's home range, the number of invasion events, and pathogen infection rates. Our first aim in this study was to estimate the specific area within the Eurasian host range from which the New Zealand and Argentinian populations originated. The two mitochondrial DNA genes produced a distinct cline in the geographic structuring in the native range of the common wasp. This cline was related to geographic distance, primarily along an east to west gradient (Fig. 4). Clines may result from geographic variation in selection pressure (e.g. Wunderle, 1981), from introgression following secondary contact (e.g. Cooke *et al.*, 1985), or simply from isolation with increasing geographic distance or other environmental variables (e.g. Brazeau *et al.*, 2013). While a clear geographic cline was observed, some haplotypes were shared in multiple geographic locations. For example, haplotypes occurring in the UK and Ireland were present in Western and Eastern Europe. The wasp collected from China was the only one to have a unique haplotype not shared with any other region. The lack of distinct haplotype boundaries within the native range suggests that

mixing or dispersal within these wasp populations might have occurred, which could have been caused by either natural or human-aided means. Estimates of natural dispersal for Vespid queen wasps range in the hundreds of meters per year, while human-aided transport of overwintering or hibernating queens likely accounts for the their movement over tens of kilometres per year (Donovan, 1991; Goodisman *et al.*, 2001; Masciocchi & Corley, 2012). Human-aided transport of queens within the native range has likely resulted in the mixing of haplotypes and could have contributed to the observed clinal pattern.

Six haplotypes were detected among the New Zealand samples and two haplotypes were observed in the Argentinian samples. Given the geographic cline in the mitochondrial DNA, these haplotypes indicate that the introduced populations in New Zealand and Argentina both originated from Western Europe. In addition, the observation of several haplotypes in Argentina and New Zealand indicates multiple successful incursions of the common wasp into both countries. Common wasp nests are founded by a single queen (Archer, 2012) and mitochondrial DNA is maternally inherited. Hence, the occurrence of multiple haplotypes within a country indicates the introduction of multiple queens into that country. Established nests with a queen and hundreds or thousands of workers are readily apparent and are thus unlikely to have been moved. It is much more likely that fertilized queens have been moved after they have sought refuge for overwintering shelter (Beggs *et al.*, 2011). Vespine queens have long been observed to hibernate or overwinter in human goods and produce, including between books in a case, in beetle holes in wood, under corrugated iron sheeting, clinging to curtains or sacking (Duncan, 1939; Thomas, 1960). Quarantine records spanning several decades in New Zealand indicate multiple interceptions of Vespid wasps from a variety countries within Europe (e.g. France, Germany, Switzerland) and also from their introduced range (e.g. Australia) (Keall, 1981; Townsend, 1984; Richardson, 1979).

Within New Zealand, isolated individual specimens of the common wasp were observed since 1921 in several widely separated locations, supporting the theory of multiple introductions (Donovan, 1983). These New Zealand incursions may have come directly from Europe, or populations established during the 1950's in nearby Australia (Anonymous, 1962) may have also contributed propagules. The haplotype discovery curve (supplementary Figure S2) indicates there are more haplotypes in New Zealand than we observed, implying that there have been additional introductions. We thus cannot exclude incursions from regions such as Asia into New Zealand or Argentina, but our data is indicative that the predominant haplotypes established have their ultimate origin from Western Europe. The pathway of these populations into Argentina is less clear. Given the genetic similarity between New Zealand and Argentina, our results cannot discount the possibility of the Argentine populations arriving via New Zealand. Chile was the likely source of the German wasp (*Vespula germanica*) in Argentina (Beggs *et al.*, 2011; Masciocchi & Corley, 2012) and the common wasp may have arrived in Argentina via a similar route. Given the highly invasive nature of these wasps, the global propagule pressure from populations is likely to increase in an exponential fashion due to an increasing number of potential propagule sources.

Wing morphology has been used as a character for differentiating intraspecific variation in related insects including honey bees (Al-Ghamdi *et al.*, 2013), with strong support from mitochondrial DNA (Nielsen *et al.*, 1999). With our wasps, the morphometric analysis showed significant morphological variation between countries and supported the Western European origin for New Zealand populations, though not for Argentine samples. We expected considerable overlap between the New Zealand population and that of Argentina given the genetic similarity between the populations. Perhaps some environmental feature can influence wasp wing morphology, as it can for damselfies (Taylor & Merriam, 1995). The

lack of overlap between these samples suggests that variation wing morphology may not be a good character for determining the origin of common wasp propagules.

The enemy release hypothesis predicts that the abundance or diversity of pathogens, parasites and predators of invasive species is reduced in the introduced range, relative to the native range, largely due to population bottlenecks during the colonization process (Keane & Crawley, 2002; Torchin *et al.*, 2003). In the present study, we assessed the prevalence of the microsporidian pathogens within both the native and the introduced range of the common wasp. Microsporidian pathogens such as *Nosema* spp. have been associated with colony collapse in honey bees (Bromenshenk *et al.*, 2010; Evans & Schwarz, 2011), and the synergistic effects of low genetic diversity and *Nosema* spp. infection are thought to cause declines in bumble bee populations of North America (Cameron *et al.*, 2011).

We observed no significant differences in the prevalence of microsporodian infections between populations of the native and introduced ranges, and thus no evidence to support the enemy release hypothesis. Wattier *et al.* (2007) similarly found no microsporidian parasite loss after invasion by an exotic amphipod. They concluded that the amphipod invasion was either massive or recurrent, enabling the microsporidian pathogen to follow its host. Multiple introductions of the common wasp have clearly occurred in New Zealand and Argentina. The multiple incursions have likely facilitated multiple microsporidian introductions, especially given the high infection rate of these pathogens within the home range of the wasps. Microsporidia may also be acquired in the new range as exemplified in our study with apparent infection *N. bombi* in the New Zealand samples, which is a pathogen of bumble bees. Recent work has demonstrated that the individual *Nosema* species are capable of infecting multiple host species and genera (Graystock *et al.*, 2013; Fürst *et al.*, 2014). Thus, pathogens already present in an invaded zone may contribute to the pathogen loading for a new invader. Furthermore, the duration since arrival into an area may be positively correlated

with increasing pathogen accumulation, because there has been more time to acquire and accumulate infections. This phenomenon has been observed in plants (Flory & Clay, 2013) and may apply to microsporidian infections in common wasp populations as well.

Wasps share the same habitat and compete for the same resources as honey bees and bumble bees (Moller & Tilley, 1989), and they also raid honey bee hives (Clapperton *et al.*, 1989). Together, these behaviours could increase the exposure of wasps to bee pathogens such as *N. apis* and *N. bombi*. Evidence of such microsporidian pathogen spillover has been recently observed between honey and bumble bees (Fürst *et al.*, 2014). The rate of microsporidian infection in wasps of up to 53% that we observed are much higher than those previously noted in wasps or bees (e.g. 7-9% in honey and bumble bees; Fürst *et al.*, 2014). Our results are of concern to apiarists and growers reliant on bumble bee pollination, as the high infection rate in wasps may result in pathogen spillback. The management of wasp populations may be a requirement in order to manage disease in bees.

The common wasp may thus be subject to pathogen acquisition, but the pathogenicity of these different microsporidian species remains unknown. Pathogens like *Nosema apis* do infect and multiply in *Vespula germanica* (F.), with the potential to kill entire wasp nests (Fantham & Porter, 1913). Our work has similarly demonstrated that *N. apis* can infect and multiply in common wasps (unpublished data). Evison *et al.* (2012) also observed microsporidian infection in common wasps. However, the presence of "pathogens" may thus not always be deleterious and perhaps can even be advantageous for their hosts. For example, one microsporidian species is thought to have little effect on its primary invasive ladybeetle host, but the pathogen has lethal effects on native ladybeetles (Vilcinskas *et al.*, 2013). This microsporidian thus appears to facilitate the invasion and spread of its host. Further, even if enemy release does occur it may not correlate with increased demographic success, for example if enemies do not limit species in the native range (Prior & Hellmann, 2013). Our

work with microsporidian infections in the common wasp does not support the enemy release hypothesis; but neither can we reject the hypothesis. Perhaps other key pathogens that are actually pathogenic, and are rarer in wasps than microsporidian infections, are absent from their invaded range.

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BIOSKETCH

Phil Lester is a Professor in the School of Biological Sciences at Victoria University of Wellington. He has been with Victoria and the Centre for Biodiversity & Restoration Ecology since 2001. His current focus with social wasp research is examining the diversity, abundance and effects of pathogens in their native and introduced range.

Author contributions: P.J.L conceived the study; M.A.M.G and E.B-R collected data and analysed the samples; P.J.L., E.B-R, M.A, J.C.C, L.D, M.M. and A.V.O contributed samples; all authors helped write the manuscript and interpret the data.

Table 1 Prevalence of microsporidian pathogen infection in the common wasp samples from the study regions. The 95% confidence intervals (CI) were obtained from a boot-strap analysis

Region	n	positive	prevalence	95% CI range
Argentina	19	7	0.368	0.158 – 0.579
Asia	2	0	0.000	0.000 – 0.000
Eastern Europe	14	5	0.357	0.143 – 0.643
New Zealand	39	21	0.538	0.385 – 0.692
Northern Europe	7	1	0.143	0.000 – 0.429
UK & Ireland	25	10	0.400	0.200 – 0.600
Western Europe	27	7	0.259	0.111 – 0.444

610 Figure 1 Origins of the *Vespula vulgaris* samples. The samples are coloured according to their % genetic similarity to one
611 or more of the specimens from New Zealand. The Chinese specimen showed the highest genetic difference (2.9% base pairs
612 difference) compared to the New Zealand samples (Chinese sample location not shown on the map).

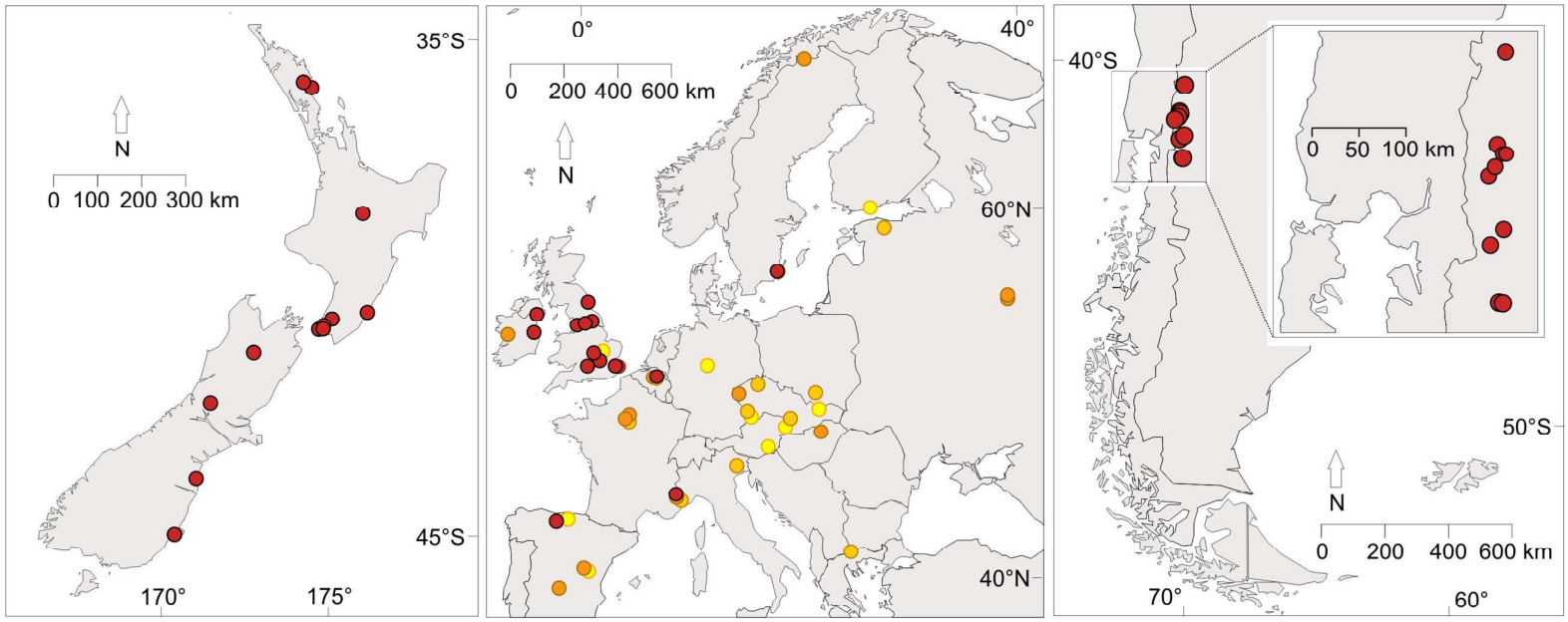

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Figure 2 Canonical discriminant analysis examining variation in wasp wing morphology within and between different countries. (i) Distances measured between nodes on wing veins (ii) Dots represent centroids of the group, while circles are 95% confidence intervals. The alphabetical script (a-b, etc.) represents axes related to individual distance measurements. We included wasps from countries from which we had 10-22 wasp samples.

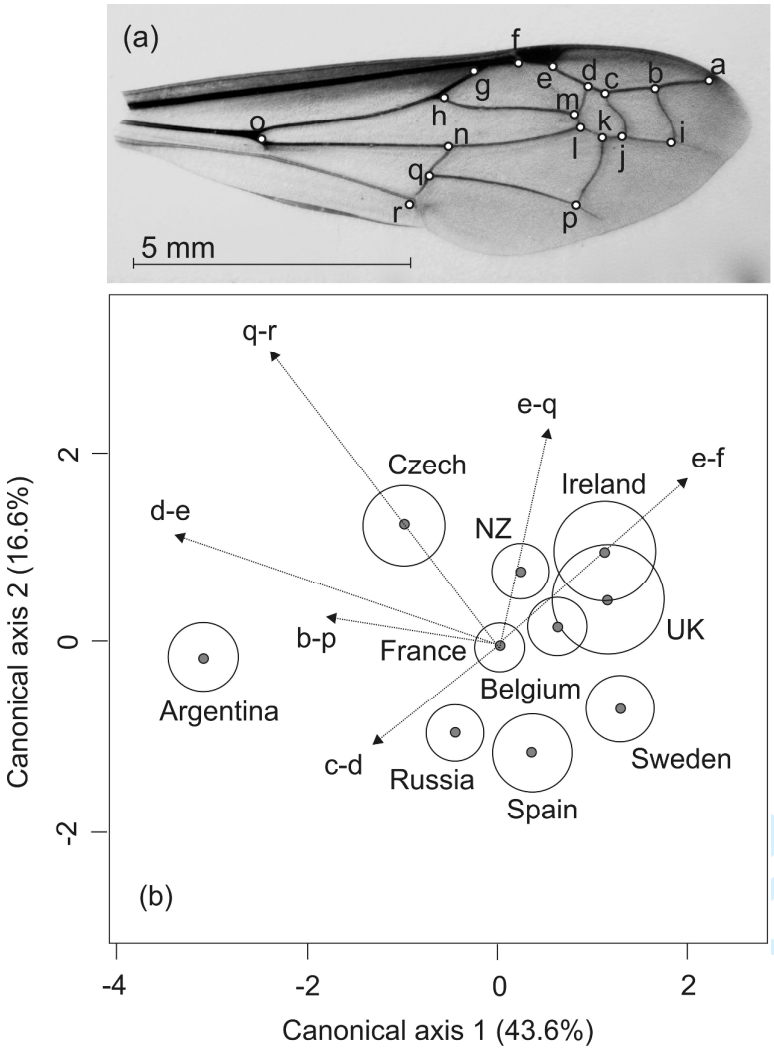


Figure 3 Maximum Composite Likelihood tree for *Vespula vulgaris* sampled throughout the native and introduced range, with *V. germanica* used to root the evolutionary tree. The tree was based on 2000 bootstraps of a Hasegawa-Kishino-Yano (HKY +G) model with gamma parameter (0.16), using a concatenated dataset of *COI* and *cytb* mtDNA sequences. The estimates of levels of support shown are bootstrap values greater than 50%. Colours identify different regional groupings (see Fig. 4). The dashed line connecting *V. vulgaris* is not to scale.

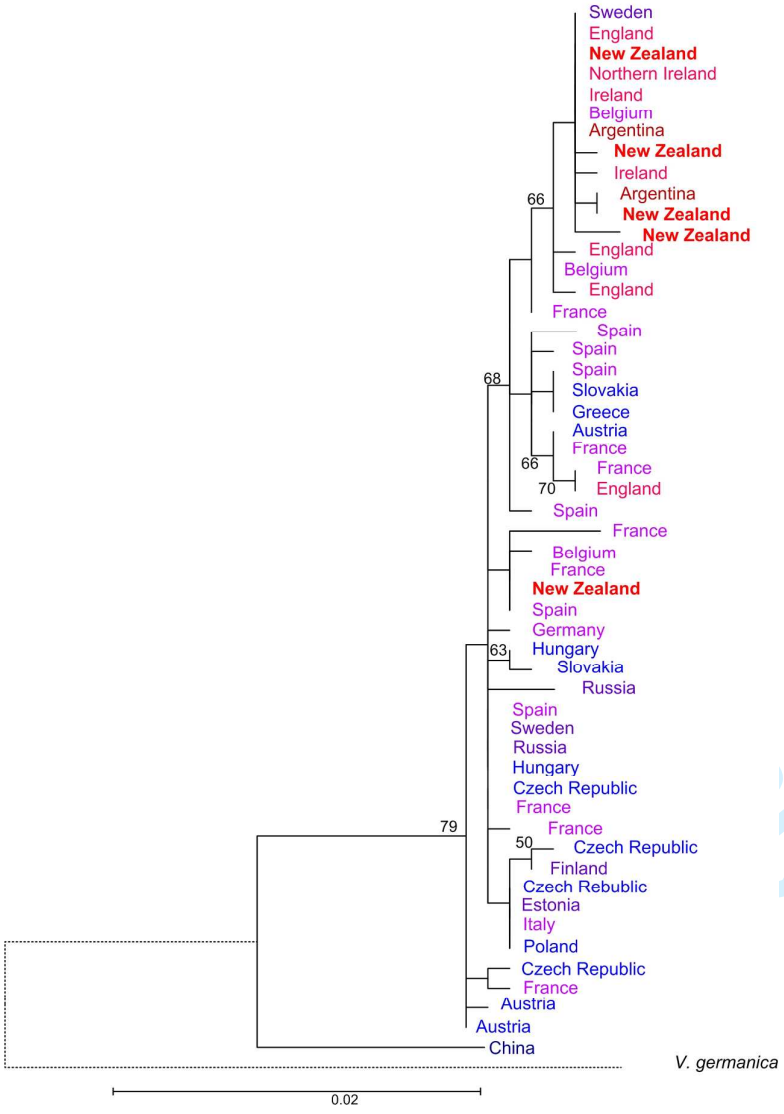
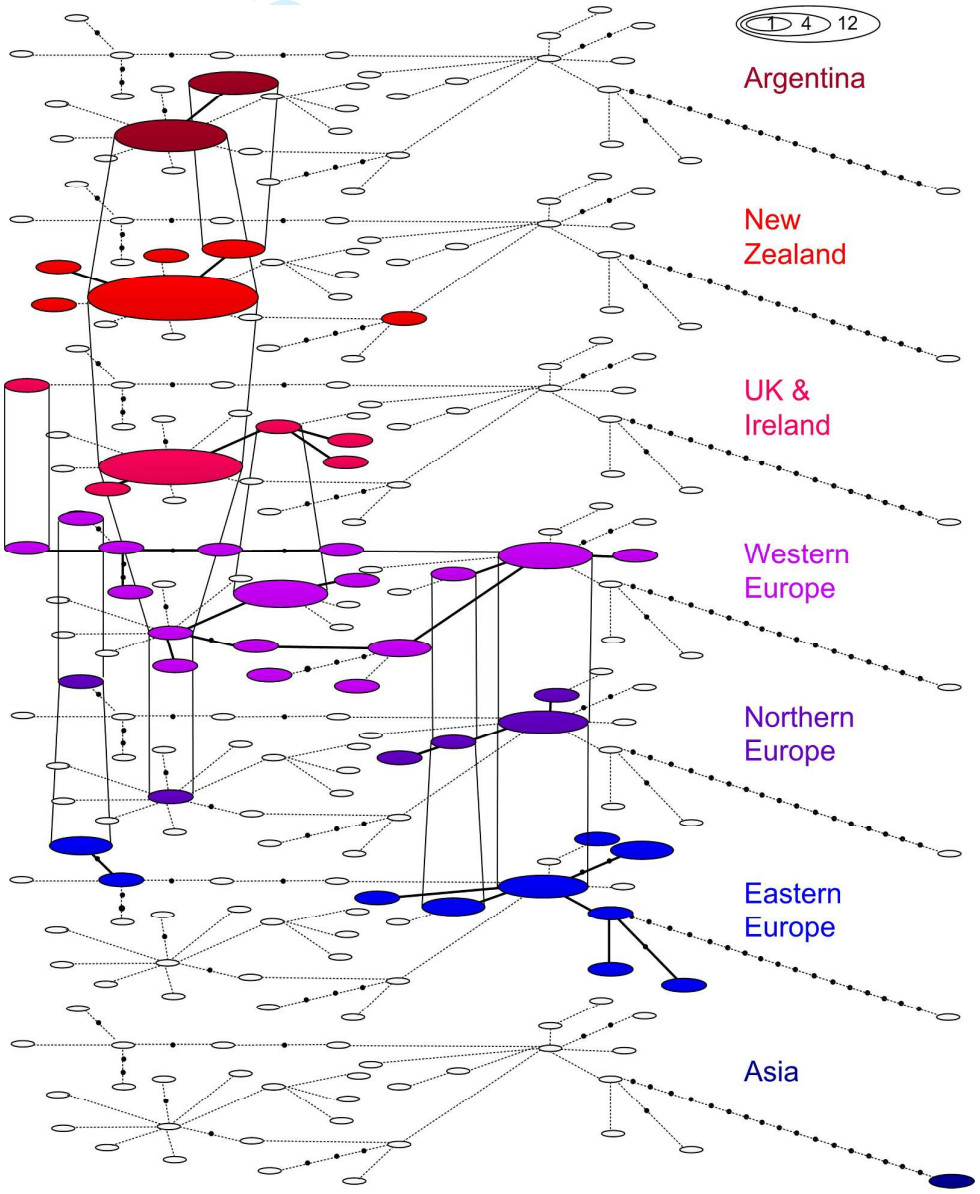


Figure 4 Spatially structured haplotype network of *Vespula vulgaris* constructed in TempNet. Lines between haplotype groups in adjacent layers indicate relationships between the groups. Filled ellipses denote a positive sample and the relative number of samples for each haplotype. Empty ellipses represent the absence of a haplotype in a particular region. Each point along the lines between haplotypes indicates a base substitution. Regional groupings are: Asia (China, n=1); Eastern Europe (Poland, Hungary, Austria, Czech Republic, Greece, Slovakia, n=16); Northern Europe (Russia, Estonia, Finland, Sweden, n=9); Western Europe (France, Belgium, Germany, Italy, Spain, n=26); UK & Ireland (England, Ireland, Northern Ireland, n=17), New Zealand (n=23), and Argentina (n=11).



Running header: Origin & enemy release in an invasive wasp

SUPPLEMENTARY MATERIAL

Running header: Origin of & enemy release in an invasive wasp

No evidence of enemy release in the invaded range of the invasive common wasp (*Vespula vulgaris*) from microsporidian pathogens

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Figure S1 Comparison of genetic distance between (and among) *Vespula vulgaris* wasps from New Zealand (closed circles), Argentina (open circles) and other regions sampled.

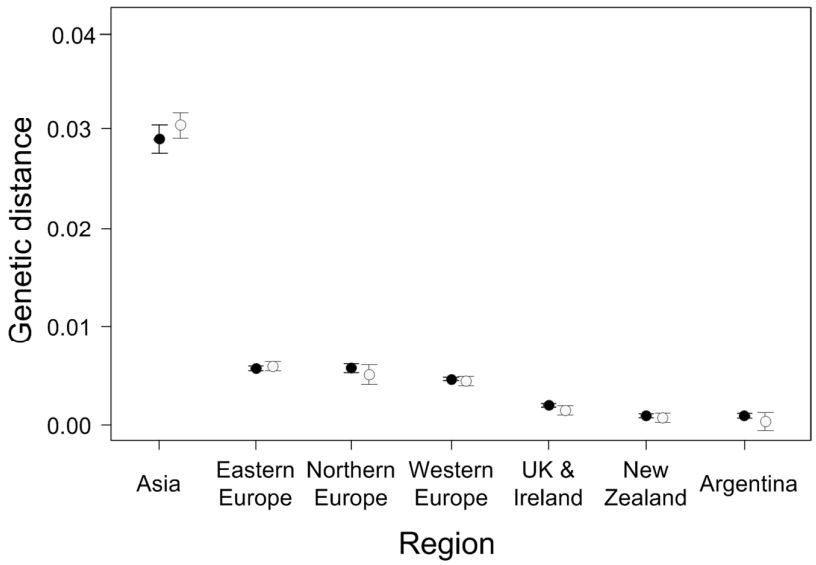

Figure S2 Haplotype discovery curve for *V. vulgaris* sampled in New Zealand

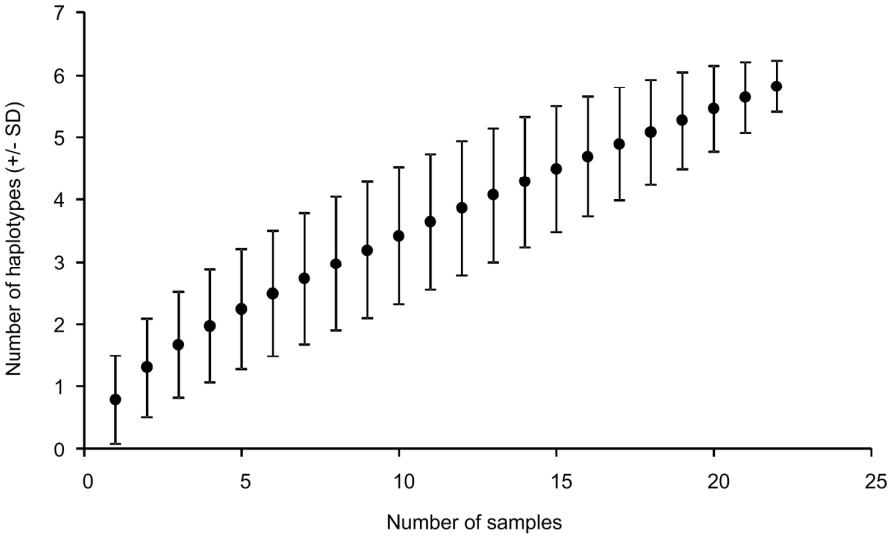

Figure S4 Relationship between genetic and geographical distance among all samples from the native range. The black dashed line represents the line of best fit (linear) and the solid orange line represents the line of best fit (smoothed using a loess function in R with a span of 0.5). The relationship between genetic and geographic distance was significant (r=0.838, p<0.001) and was also significant when the sample from China (i.e. the most distant sample) was removed (r=0.072, p<0.001).

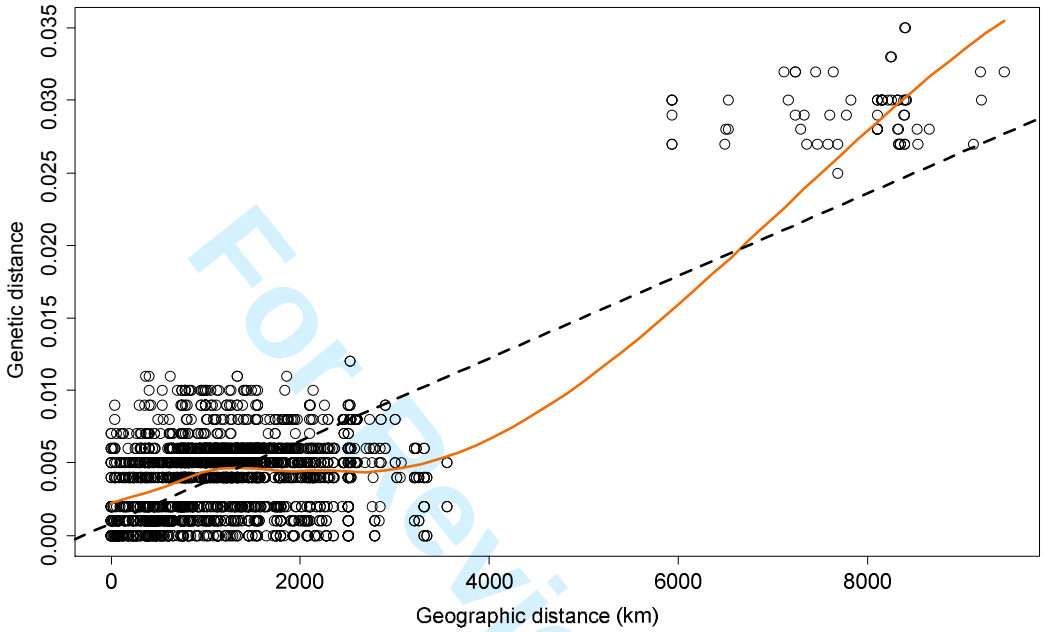

Table S1 Sampling origins and approximate collection date for *Vespula vulgaris* and the single *V. germanica* used in this study. Wasps were typically collected while foraging. When wasps were collected directly from nests, only one individual per nest was used in the analysis

Country	Locale	Latitude	Longitude	Collection date	Submitter
Argentina	Chubut	-42.509	-71.432	9 February 2013	M. Masciocchi
Argentina	Chubut	-42.507	-71.425	9 February 2013	M. Masciocchi
Argentina	Chubut	-41.918	-71.553	1 March 2012	M. Masciocchi
Argentina	Chubut	-41.918	-71.553	1 March 2012	M. Masciocchi
Argentina	Río Negro	-41.850	-71.417	18 February 2013	M. Masciocchi
Argentina	Río Negro	-41.348	-71.617	6 February 2013	M. Masciocchi
Argentina	Río Negro	-41.271	-71.508	6 February 2013	M. Masciocchi
Argentina	Río Negro	-41.161	-71.411	5 February 2013	M. Masciocchi
Argentina	Río Negro	-41.119	-71.402	17 February 2013	M. Masciocchi
Argentina	Río Negro	-41.099	-71.447	5 February 2013	M. Masciocchi
Argentina	Neuquén	-40.161	-71.358	15 March 2013	M. Masciocchi
Austria	Styria	47.071	15.440	3 September 2012	H. Kovac
Austria	Mühlviertel	48.585	14.035	13 July 2007	L. Dvořák
Austria	Mühlviertel	48.708	13.854	13 July 2007	L. Dvořák
Belgium	Leuven	50.797	4.985	12 September 2012	A. Van Oystaeyen
Belgium	Leuven	50.797	4.985	12 September 2012	A. Van Oystaeyen
Belgium	Leuven	50.842	4.671	18 September 2012	A. Van Oystaeyen
Belgium	Leuven	50.885	4.659	25 July 2012	A. Van Oystaeyen
Belgium	Leuven	50.885	4.659	25 July 2012	A. Van Oystaeyen
Belgium	Leuven	50.885	4.659	25 July 2012	A. Van Oystaeyen
Belgium	Leuven	50.926	4.985	21 September 2012	A. Van Oystaeyen
China	Shanxi	35.302	111.671	1 July 2012	N.T.P. Lien
Czech Republic	Modrava	49.023	13.495	12 August 2005	L. Dvořák
Czech Republic	Mariánské Lázně	49.965	12.701	1 September 2012	L. Dvořák
Czech Republic	Mariánské Lázně	49.965	12.701	1 September 2012	L. Dvořák
Czech Republic	Mariánské Lázně	49.965	12.701	1 September 2012	L. Dvořák
Czech Republic	Chudolazy	50.474	14.479	31 July 2004	L. Dvořák
Estonia	Järvamaa, Koeru	58.965	26.026	19 July 2006	L. Dvořák
Finland	Helsinski-Herttoniemi	60.197	25.017	6 September 2008	L. Dvořák
France	Saint Martin-Vesubie	44.117	7.287	1 July 2009	A. Perrard
France	Saint-Dalmas-le Selvage	44.285	6.888	1 July 2009	A. Perrard
France	Saint-Dalmas-le Selvage	44.293	6.824	1 July 2009	A. Perrard
France	Meyronnes	44.475	6.797	1 July 2011	A. Perrard
France	Marchais	48.447	2.393	11 July 2007	L. Dvořák
France	Marchais	48.447	2.393	11 July 2007	L. Dvořák
France	Bonnelles	48.618	2.028	11 July 2007	L. Dvořák
France	Vincennes	48.833	2.421	6 October 2012	A. Perrard
France	Vincennes	48.833	2.421	6 October 2012	A. Perrard

Country	Locale	Latitude	Longitude	Collection date	Submitter
Germany	Barterode	51.548	9.743	11 July 2007	L. Dvořák
Greece	Sultanitsa	41.317	23.201	11 July 2007	L. Dvořák
Hungary	Felsotarkany	47.899	20.385	20 June 2010	G. Broad
Hungary	Felsotarkany	47.899	20.385	20 June 2010	G. Broad
Hungary	Felsotarkany	47.899	20.385	20 June 2010	G. Broad
Ireland	Creglucas	53.225	-8.865	10 July 2006	L. Dvořák
Ireland	County Kildare	53.340	-6.538	1 September 2012	R. O'Toole
Italy	Pordenone	46.031	12.494	7 September 2007	L. Dvořák
Mongolia	Central Aimak	47.833	107.400	13 July 2002	L. Dvořák
New Zealand	Otago	-45.890	170.500	1 March 2013	T. Harris
New Zealand	Otago	-45.890	170.500	1 March 2013	T. Harris
New Zealand	Otago	-45.890	170.500	1 March 2013	T. Harris
New Zealand	Otago	-45.890	170.500	10 March 2013	T. Harris
New Zealand	South Canterbury	-44.632	171.141	14 February 2013	T. Harris
New Zealand	Canterbury	-42.945	171.566	28 February 2013	B. Brown
New Zealand	Nelson	-41.808	172.851	1 April 2012	P.J. Lester
New Zealand	Nelson	-41.803	172.846	20 February 2011	P.J. Lester
New Zealand	Nelson	-41.803	172.846	20 February 2011	P.J. Lester
New Zealand	Wellington	-41.286	174.776	1 May 2012	J. Barnard
New Zealand	Wellington	-41.286	174.776	1 May 2012	J. Barnard
New Zealand	Wellington	-41.278	174.909	1 April 2013	E. Brenton-Rule
New Zealand	Wellington	-41.194	174.923	18 April 2012	P.J. Lester
New Zealand	Wellington	-41.051	175.171	1 February 2012	R. Barbieri
New Zealand	Wellington	-41.051	175.171	1 February 2012	R. Barbieri
New Zealand	Wellington	-41.051	175.171	1 February 2012	R. Barbieri
New Zealand	Wellington	-41.051	175.171	1 February 2012	R. Barbieri
New Zealand	Castlepoint	-40.900	176.217	17 February 2013	P.J. Lester
New Zealand	Taupo	-38.683	176.083	23 February 2013	E. Brenton-Rule
New Zealand	Northland	-35.850	174.567	20 April 2012	F.R. Schnitzler
New Zealand	Northland	-35.850	174.567	20 April 2012	F.R. Schnitzler
New Zealand	Northland	-35.725	174.324	2 May 2012	B. Thompson
New Zealand	Northland	-35.725	174.324	2 May 2012	B. Thompson
Northern Ireland	County Down	54.349	-6.270	1 September 2012	S. Curran
Poland	Góra Pychowicka	50.031	19.883	16 August 2006	L. Dvořák
Russia	Moscow	55.215	37.913	24 August 2012	A.V. Antropov & N.A.Khrustalyova
Russia	Moscow	55.216	37.911	17 August 2012	A.V. Antropov & N.A.Khrustalyova
Russia	Moscow	55.217	37.902	24 August 2012	A.V. Antropov & N.A.Khrustalyova
Russia	Moscow	55.409	37.906	24 August 2012	A.V. Antropov & N.A.Khrustalyova
Slovakia	Dlhé Rovné	48.636	17.530	4 August 2004	L. Dvořák
Slovakia	Starý Smokovec	49.134	20.212	26 September 2006	L. Dvořák
Spain	Los Cortijos	40.167	1.483	16 August 2007	L. Dvořák
Spain	Los Cortijos	40.167	1.483	16 August 2007	L. Dvořák
Spain	Teruel	40.240	-1.410	6 October 2012	L. Castro
Spain	Teruel	40.408	1.444	1 October 2012	L. Castro

Country	Locale	Latitude	Longitude	Collection date	Submitter
Spain	Palencia	42.996	-4.531	10 August 2012	L. Castro
Spain	Burgos	43.102	-3.343	23 September 2012	L. Castro
Sweden	Lappland, Abisko	68.355	18.817	15 August 2006	L. Dvořák
UK	Berkshire	51.517	-1.517	11 August 2006	E.G. Chambers
UK	Harpendan	51.816	-0.361	1 September 2010	M. Archer
UK	Harpendan	51.816	-0.361	1 September 2010	M. Archer
UK	Bucks	52.229	-0.926	17 August 2011	G. Broad
UK	Bucks	52.229	-0.926	17 August 2011	G. Broad
UK	Cambridgeshire	52.325	-0.073	22 June 2013	H. Berman
UK	Lancashire	53.749	-2.488	1 September 2012	M. Archer
UK	Herts	53.850	-1.725	7 January 2012	G. Broad
UK	York	53.962	-1.082	8 October 2012	M. Archer
UK	Hunts	55.020	-1.460	3 June 2005	G. Broad
UK	Hunts	55.020	-1.460	3 June 2005	G. Broad
UK	Hunts	55.020	-1.460	3 June 2005	G. Broad
UK	Lambourn Downs	51.489	1.425	10 August 2006	E.G. Chambers
UK	Reading	51.524	1.093	10 August 2006	E.G. Chambers
Vespula germanica					
New Zealand	Auckland	-36.972	174.840	25 May 2012	F.R. Schnitzler

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Table S2 The maximum likelihood fits for the five best-fitting models of the 24 different nucleotide substitution models calculated in MEGA 5.1 for the separate *cytb* and *COI* datasets and concatenated dataset (852 bases). Model abbreviations: HKY=Hasegawa-Kishino-Yano; TN93=Tamura-Nei; T92=Tamura 3-parameter. The best-fitting substitution model was selected based on Bayesian Information Criterion scores (BIC). For each model the corrected Akaike Information Criterion value (AICc), Maximum Likelihood value (lnL), and the number of parameters are also presented. Models with the lowest BIC, AICc and lnL scores describe the substitution pattern the best. Variable model parameters included non-uniformity of evolutionary rates among sites (a discrete Gamma distribution (+G) with 5 rate categories) and the assumption that a certain fraction of sites are evolutionarily invariable (+I). Where applicable the estimates of +G and +I are shown. The number of parameters in the model (#), and the assumed or estimated values of transition/transversion bias (R) are also shown. MEGA also calculated nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair (data not shown)

Dataset	Model	#	BIC	AICc	lnL	+I	+G	R
Composite	HKY+G	110	4867	3907	-1843	-	0.16	2. 09
	HKY+G+I	111	4874	3901	-1841	0.57	0.63	2.21
	TN93+G	111	4878	3909	-1843	-	0.16	2.09
	T93+I	108	4881	3912	-1844	0.75	-	2.11
	TN92+G	112	4883	3940	-1861	-	0.15	2.22
COI	T92+I	110	2856	1972	-875	0.82	-	3.69
	T92+G	110	2857	1973	-876	-	0.05	3.19
	HKY+G	112	2862	1962	-868	-	0.06	2.75
	HKY+I	112	2862	1962	-868	0.81	-	2.83
	T92+G+I	111	2864	1972	-874	0.70	0.51	3.75
cytb	HKY	111	2896	2000	-888	-	-	1.63
	HKY+G	112	2900	1997	-886	-	0.58	1.77
	T92	109	2901	2022	-901	-	-	1.63
	TN93	112	2903	1999	-887	-	-	1.65
	HKY+I	112	2905	2001	-888	0.12	-	1.65

Table S3 Results of GLM analysis comparing: a) the genetic distance between the New Zealand samples and all other regions; and b) the genetic distance between the Argentina samples and all other regions

	Region	difference	t	p
a) New Zealand				
	Argentina	1.0 times greater	0.78	0.436
	UK and Ireland	2.0 times greater	6.72	<0.001
	Western Europe	5.5 times greater	17.54	<0.001
	Northern Europe	7.2 times greater	19.48	<0.001
	Eastern Europe	7.1 times greater	19.92	<0.001
	Asia	38.5 times greater	34.33	<0.001
b) Argentina				
	New Zealand	1.4 times greater	1.30	0.195
	UK and Ireland	3.2 times greater	4.78	<0.001
	Western Europe	8.9 times greater	9.18	<0.001
	Northern Europe	9.8 times greater	9.21	<0.001
	Eastern Europe	11.9 times greater	10.41	<0.001
	Asia	60.4 times greater	16.66	<0.001

Table S4 Matrix of pairwise genetic distances between individuals using the Tamura-Nei model (Tamura and Nei 1993).
(Refer to Lester et al Origin of Vespula vulgaris SUP_MAT.xlsx)

Table S5 PCR results from triplicate PCRs including positives and negatives. Positive samples were sequenced and BLASTn searched to confirm they were microsporidia

Region	PCR1	PCR2	PCR3	Total positive
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	0	0
Argentina	0	0	1	1
Argentina	0	1	0	1
Argentina	0	0	1	1
Argentina	0	0	0	0
Argentina	0	1	0	1
Argentina	1	1	0	2
Argentina	1	0	0	1
Argentina	1	0	0	1
Argentina	0	0	0	0
Argentina	0	0	0	0
China	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	0	0	0
Eastern Europe	0	1	1	2
Eastern Europe	0	1	0	1
Eastern Europe	0	1	0	1
Eastern Europe	0	0	0	0
Eastern Europe	1	0	0	1
eastern_europe	1	0	0	1
Eastern Europe	0	0	1	1
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	1	0	1
New Zealand	1	1	0	2

Region	PCR1	PCR2	PCR3	Total positive
New Zealand	0	1	1	2
New Zealand	1	0	0	1
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	1	1	2
New Zealand	0	1	1	2
New Zealand	0	0	0	0
New Zealand	1	1	1	3
New Zealand	0	1	1	2
New Zealand	0	1	0	1
New Zealand	0	0	0	0
New Zealand	0	0	1	1
New Zealand	0	0	1	1
New Zealand	0	1	0	1
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	1	1	2
New Zealand	0	1	1	2
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	1	0	1
New Zealand	1	0	0	1
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	1	0	1
New Zealand	0	1	0	1
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	0	0	0
New Zealand	0	1	0	1
New Zealand	0	1	0	1
New Zealand	0	0	0	0
New Zealand	0	0	0	0
Northern Europe	0	0	0	0
Northern Europe	0	0	0	0
Northern Europe	0	0	0	0
Northern Europe	0	0	0	0
Northern Europe	0	0	0	0
Northern Europe	0	0	0	0
Northern Europe	1	0	0	1
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0

Region	PCR1	PCR2	PCR3	Total positive
UK & Ireland	0	0	0	0
UK & Ireland	0	1	0	1
UK & Ireland	0	1	1	2
UK & Ireland	0	1	1	2
UK & Ireland	0	1	0	1
UK & Ireland	0	0	0	0
UK & Ireland	0	0	1	1
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	1	0	0	1
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	0	0	1	1
UK & Ireland	0	0	0	0
UK & Ireland	0	0	0	0
UK & Ireland	1	0	1	2
UK & Ireland	1	0	0	1
UK & Ireland	1	0	0	1
UK & Ireland	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	1	1
Western Europe	1	0	0	1
Western Europe	0	1	0	1
Western Europe	0	1	0	1
Western Europe	0	0	0	0
Western Europe	0	1	0	1
Western Europe	0	0	1	1
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0

Region	PCR1	PCR2	PCR3	Total positive
Western Europe	0	0	0	0
Western Europe	0	1	1	2
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0
Western Europe	0	0	0	0