International Journal for Parasitology xxx (xxxx) xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Long-term presence of emerging pathogens in island honey bee colonies

Micaela Sánchez-Aroca [a], Irene Muñoz [b], Pilar De la Rúa [a], Vicente Martínez-López [a,*]

[a] Department of Zoology and Physical Anthropology, University of Murcia 30100 Murcia, Spain
[b] Department of Biodiversity, Ecology and Evolution, Complutense University of Madrid 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 2 July 2025
Received in revised form 22 December 2025
Accepted 23 December 2025
Available online xxxx

Keywords:
Apis mellifera
Canary Islands
Temporal analysis
Lotmaria passim
Apicystis bombi

ABSTRACT

Honey bees are recognised as the primary pollinators of most agricultural crops and numerous wild plant species worldwide. However, the colony losses reported over recent decades pose a serious threat to this essential ecosystem service. The spread of pathogens has been identified as a significant factor contributing to the decline of honey bee populations. Consequently, there is a considerable interest in expanding our knowledge on the prevalence of emerging pathogens on honey bee colonies, particularly trypanosomatids and neogregarines. Herein, we conducted a spatio-temporal analysis of the prevalence of trypanosomatids (*Lotmaria passim* and *Crithidia mellificae*) and a neogregarine (*Apicystis bombi*) in honey bee populations across the Canary Islands sampled over a 20-year period (1998–2017). We also examined whether pathogen prevalence was associated with the introduction of foreign honey bee queens to the islands and the implementation of a conservation programme of the local Canarian black honey bee. Our results indicate that *L. passim* has been present in the Canary Islands since at least 1998, whereas *C. mellificae* was not detected. This finding represents the earliest known global record of the *L. passim* worldwide. *Apicystis bombi* was found on several islands during the study period, though at low frequency. The prevalence of *L. passim* did not exhibit any correlation with the introduction of foreign honey bee queens, unlike other pathogens and parasites such as *Nosema ceranae* and *Varroa destructor*. Notably, the implementation of long-standing conservation measures in La Palma was associated with a higher prevalence of *L. passim* compared to Gran Canaria. These results suggest that *L. passim* may have been present in the Canary Islands prior to the introduction of foreign honey bees in recent decades. Further analyses of historical samples from additional regions, particularly from geographically isolated areas such as islands, are necessary to untangle the spread history of *L. passim* in honey bee populations.

1. Introduction

The honey bee is considered an essential pollinator for crops and wild flora (Klein et al., 2007; Ollerton et al., 2011), contributing to human health and nutrition (Delaplane and Mayer, 2000; Gallai et al., 2009). In recent decades, there have been significant losses of honey bee colonies due to different factors (De la Rúa et al., 2009; Jaffé et al., 2010; Meana et al., 2017) among which the spread of pathogens and parasites is prominent (Goulson et al., 2015; Ratnieks and Carreck, 2010). Humans have particularly contributed to the spread of honey bee pathogenic agents through the trade of colonies and migratory beekeeping (Ellis and Munn, 2005; Goulson et al., 2015; Jara et al., 2020; Martínez-López et al., 2022a). For instance, the ectoparasitic mite *Varroa destructor* and the microsporidia pathogen *Vairimorpha* (*Nosema*) *ceranae* (Bojko et al., 2025), both originally found in the Asian honey bee (*Apis cerana*), were able to infest the Western honey bee (*Apis mellifera*) and spread worldwide, which has resulted in significant honey bee colony losses (Martín-Hernández et al., 2018; Rosenkranz et al., 2010). Indeed, both organisms are now regarded as emerging pathogens of honey bees, with *N. ceranae* even being detected in other managed and wild pollinators (Grupe and Quandt, 2020; Martínez-López et al., 2022b). Therefore, there is considerable interest in understanding the impact of other potential emerging pathogens on honey bee colonies, such as trypanosomatids and neogregarines.

Research on the prevalence of trypanosomatids in honey bee colonies have increased in recent decades due to their potential as drivers of colony mortality (Cepero et al., 2014; Cornman et al., 2012; MacInnis et al., 2025; Ravoet et al., 2013). *Crithidia mellificae* was the first trypanosomatid described in honey bees (Langridge and McGhee, 1967), but molecular analyses revealed a new species,

* Corresponding author.
E-mail address: vicente.martinez2@um.es (V. Martínez-López).

https://doi.org/10.1016/j.ijpara.2025.104767

Lotmaria passim (Schwarz et al., 2015a), which showed higher prevalence in colonies than *C. mellificae* (Ravoet et al., 2015; Schwarz et al., 2015a). Indeed, *L. passim* has been the predominant trypanosomatid found in honey bees in Asia (Japan, Morimoto et al., 2013; Ravoet et al., 2015), Europe (Belgium and Switzerland, Ravoet et al., 2015; Serbia, Stevanovic et al., 2016; Czech Republic, Mráz et al., 2021; Italy, Bordin et al., 2022; Ribani et al., 2021), Macaronesian islands (Azores and Madeira, Aguado-López et al., 2023), North America (USA, Williams et al., 2019) and South America (Chile, Arismendi et al., 2016; Argentina, Chile and Uruguay, Castelli et al., 2019). *Lotmaria passim* and *C. mellificae* impair the health of honey bees by reducing their life expectancy and producing changes in their bacteriome and individual physiology (Gómez-Moracho et al., 2020; Hubert et al., 2017; Liu et al., 2020; MacInnis et al., 2025; Schwarz et al., 2015b). However, the life cycle, mechanisms of pathogenesis, and modes of host-to-host transmission are still not fully understood (Buendía-Abad et al., 2022).

With regard to neogregarines, *Apicystis bombi*, previously classified as *Mattesia bombi* (Macfarlane et al., 1995), causes serious physical and behavioural alterations in *Bombus* spp. (Jones and Brown, 2014; Rutrecht and Brown, 2008; Schmid-Hempel, 2001) although its prevalence is usually low (Lipa and Triggiani, 1996). *Apicystis bombi* has also been detected in honey bees (Lipa and Triggiani, 1992; Plischuk et al., 2011), but its impact on honey bee health is not clear (Plischuk et al., 2011). On the other hand, the prevalence of neogregarines in honey bee colonies is generally lower than that of trypanosomatids (Cepero et al., 2014; Figueroa et al., 2021).

The question of whether these trypanosmatids and neogreagrines have been associated with honey bees prior to the spread of other emerging pathogens, such as *V. destructor* or *N. ceranae*, linked to the honey bee trade, remains unresolved. For instance, Aguado-López et al. (2023) conducted a study on the prevalence of trypanosomatids on a number of islands within the Madeira and Azores archipelagos. Their findings indicated the presence of *L. passim* on islands devoid of *V. destructor* and/or *N. ceranae*, thereby suggesting that this trypanosomatid may have been associated with honey bees prior to the dissemination of these emerging pathogens. Nevertheless, the verification of this hypothesis requires further data and more studies in isolated areas, such as islands, where honey bees can be easily traced, are required. On the other hand, the initial global detections of *L. passim* date back to 2007 in Serbia (Stevanovic et al., 2016) and South America (Castelli et al., 2019), suggesting that analysing historical samples would facilitate the investigation of the past distribution of the pathogen and, thereby, determine whether it has been linked to honey bees prior to the spread of other emerging pathogens.

A considerable number of studies have been conducted on the genetic diversity, structure, and integrity of honey bee populations of the Canary Islands. While these studies have demonstrated the existence of a particular ecotype of black honey bee (De la Rúa et al., 1998; Miguel et al., 2015), they have also revealed the introduction of honey bee queens of European origin in this Atlantic archipelago (De la Rúa et al., 2002; Muñoz et al., 2013; Muñoz and De la Rúa, 2012). Conversely, studies on the pathology of honey bees in the Canary Islands are scarce, which hampers our ability to track the spread of pathogens through the archipelago. In this context, Muñoz et al. (2014) assessed the relationship between the introduction of foreign honey bee queens on the Canary Islands and the presence and spread of *N. ceranae* on the archipelago. Their findings indicated a positive association between the introduction of honey bee queens and the subsequent increase in the prevalence of *N. ceranae* across the islands during the study period. This kind of information is increasingly in demand due to the impact of pathogens on honey bee colony mortality, especially because variation in pathogen virulence and potential local adaptations of honey bee populations can lead to markedly different disease outcomes (Goulson et al., 2015; Lee et al., 2015). On the other hand, conservation programmes aiming to safeguard the genetic integrity of the black Canarian honey bee (Consejeria de Agricultura, Ganadería, Pesca y Alimentación, 2001; Consejería de Agricultura, Ganadería, 2014) could potentially influence the spread of pathogens in the islands where applied. Among the conservation measures implemented was the breeding and dispersal of Canarian honey bee queens. If the health of these queens in terms of pathogen presence is not monitored, it could lead to the unintentional spread of pathogens and ultimately compromise the success of the conservation programme.

Herein, we perform a spatio-temporal analysis of the prevalence of trypanosomatids (*L. passim* and *C. mellificae*) and a neogregarine (*A. bombi*) in honey bee populations from the Canary Islands spanning 20 years (1998–2017). Our primary goal is to assess the presence and spread of the aforementioned pathogens throughout the study period and across islands to check whether these pathogens were already present in historical samples and whether their presence on the islands, if any, is correlated with the introduction of foreign honey bee queens. Understanding this is essential because both pathogen virulence and potential local adaptations of island honey bee populations can influence disease dynamics and colony losses. Therefore, establishing the historical timing and pathways of pathogen presence provides critical context for interpreting current honey bee health trends. Differences in the management of honey bee colonies on the islands, particularly with regard to the introduction of foreign honey bee queens and the implementation of conservation measures for the endemic ecotype of the black Canarian honey bee, may lead to variations in the prevalence of these pathogens.

2. Material and methods

2.1. Honey bee surveys

Worker honey bees analysed corresponded to different surveys carried out over a period of 20 years (Table 1) to characterise the honey bee colonies present on the Canary Islands and the level of introduction of foreign honey bees. Forty-eight colonies were sampled in 1998 and 139 during 2008, 2010, and 2011 on Tenerife, Gran Canaria, El Hierro, La Gomera and La Palma. The most recent survey was conducted in 2016 and 2017 on the islands of Gran Canaria (53 colonies) and La Palma (53 colonies). All worker honey bees were taken from the inner frames of the hives and preserved in absolute ethanol at −20 °C until processing in the laboratory.

2.2. Molecular detection of pathogens

Although the primary aim of the surveys analysed here was to investigate questions of conservation genetics, DNA-based techniques allowed for the analysis of the presence of pathogens and temporal changes in their prevalence in historical samples preserved in ethanol. In this study, protocols by de Miranda et al. (2021) were followed.

The abdomens of the available worker honey bees from each colony were separated and transferred to 50 mL Falcon® tubes. After two consecutive washes with 30 mL of distilled water followed by two additional washes with 30 mL milli-Q water in which the tubes were hand-shaken for 1 min, abdomens were then macerated in 5 mL of milli-Q water in the Stomacher® 80 Biomaster homogeniser (Seward, West Sussex) or with the TissueRuptor® blender (QIAGEN) using Stomacher® philtre bags (Seward). They were then centrifuged at 4 °C for 10 min at 1500 rpm, the supernatant was discarded, and the pellet was resuspended in 1 mL of distilled water.

Table 1
Number (*N*) of honey bee colonies sampled in each Canary Island, number of colonies positive for *Lotmaria passim* and *Apicystis bombi* and prevalence of *L. passim* per island in the different surveys. Pathogen prevalence for *A. bombi* is not shown due to the low presence.

Year	Island	Colonies (*N*)	*L. passim* positive colonies	*A. bombi* positive colonies	Prevalence *L. passim* (%)
1998	Tenerife	24	1	1	4.2
	Gran Canaria	5	5	0	100.0
	El Hierro	4	0	0	0.0
	La Gomera	5	0	0	0.0
	La Palma	10	5	0	50.0
Summary		48	11	1	22.9
2008	Tenerife	12	3	0	25.0
	Gran Canaria	10	1	0	10.0
	El Hierro	7	0	0	0.0
	La Gomera	7	1	1	14.3
	La Palma	12	3	0	25.0
Summary		48	8	1	16.7
2010	Gran Canaria	16	3	0	18.8
	La Gomera	14	7	0	50.0
	La Palma	16	10	2	62.5
Summary		46	20	2	43.5
2011	Gran Canaria	19	10	2	52.6
	El Hierro	26	5	1	19.2
Summary		45	15	3	33.3
2016	Gran Canaria	53	9	0	16.9
2017	La Palma	53	32	0	60.4
Overall summary		293	95	7	32.42

DNA extractions of the samples from the previous surveys were performed using BS96 DNA Tissue extraction protocol in a BioSprint workstation (Qiagen) according to manufacturer's instructions, whereas samples of the more recent surveys were extracted following Ivanova et al. (2006). Trypanosomatid and neogregarine pathogens were PCR-amplified with primers described by Meeus et al. (2010). These primers target the 16S ribosomal gene and amplify a fragment of 417 base pairs (bp) from trypanosomatids and 260 bp from neogregarine species. PCR reactions were performed in a 25-μL volume containing 2.5 μL of the DNA template (20 ng/μl). The PCR temperature profile consisted of an initial denaturation at 95 °C for 10 min, followed by 35 cycles at 95 °C for 30 s, 57 °C for 30 s and 72 °C for 45 s, and a final extension of 7 min at 72 °C. Extraction and PCR negative controls and a positive control were included in all the analyses and ran in parallel.

The amplicons of each pathogen-positive sample were sent for sequencing in both directions to SECUGEN S.L. The sequences were aligned with the MEGA7 programme (Kumar et al., 2016) and the identity of the species was confirmed through the BLAST tool in GenBank.

2.3. Haplotype determination for honey bee colony characterisation

Mitochondrial DNA is maternally inherited (Meusel and Moritz, 1993) and is therefore shared by all drones and workers in the same colony. Analysis of a single worker allows the characterisation of all individuals in the same colony (Meixner et al., 2013). Sequence variation of the intergenic region located between the tRNA genes and the cytochrome oxidase subunit II (*cox2*) of the mitochondrial DNA (Garnery et al., 1993) was used to determine the haplotype and the evolutionary lineage/sublineage present in each honey bee colony. This intergenic region contains two types of components, P and Q (Cornuet et al., 1991). The P sequence has three forms with different sizes based on the presence of several indels: P with 54 bp, P_0 with 67 bp, or P_1 with 50 bp. The Q sequence can be repeated up to four times and is 194–196 bp long. The number and combination of these components are unique to the four evolutionary lineages of *A. mellifera* subspecies and three African sub-lineages.

The sub-lineage A_{III} of the African lineage with Atlantic distribution, which includes populations from the Canary Islands (De la Rúa et al., 2001, 1998), Azores, and Madeira (De la Rúa et al., 2006) as well as northern Portugal (Pinto et al., 2013, 2012), is identified by the P_1 sequence and the presence of up to four Q sequences (as reviewed by Chávez-Galarza et al., 2017). Subspecies from eastern and central Europe, including the globally imported *A. m. ligustica* and *A. m. carnica* among others, that are included in lineage C have only one Q copy and no P sequence. On the other hand, subspecies of the western European M lineage, such as *A. m. mellifera* and northern *A. m. iberiensis*, typically have a combination of P and up to four Q sequences. African subspecies and southern populations of *A. m. iberiensis* (lineage A) also have one to four Q sequences but one P_0 sequence. In this study, queens were classified as foreign if they had maternal ancestry of C or M lineage, and native if they had African ancestry.

Data on the honey bee haplotype and evolutionary lineage/sublineage of samples from the previous surveys (from 1998 to 2011) have been published (De la Rúa et al., 2001; Muñoz et al., 2013). DNA extraction from the recent surveys (2016 and 2017) was performed using two legs from one worker honey bee per colony following standard methods (Evans et al., 2013). The intergenic region was amplified using MyTaq™ kit (Bioline, London, UK) and E2 and H2 primers (Garnery et al., 1993). Briefly, DNA was amplified in a thermocycler PTC 100 (MJ Research) in a total volume of 12.5 μL, 2.5 μL of the DNA template (20 ng/μl), with the following programme: 94 °C (5 min); 35 cycles of a 45 s denaturation at 94 °C, a 45 s elongation at 48 °C, a 60 s extension at 62 °C; and a final extension step at 65 °C for 20 min. The success of the PCR reactions was verified by electrophoresis in a 3 % agarose gel. Amplified fragments were submitted for sequencing (Secugen S. L., Madrid, Spain) using an ABI® PRISM 310 sequencer (Applied Biosystems, Foster City, CA, USA) with E2 and H2 primers to determine the composition of the $RNAt^{leu}$-*cox2* intergenic region and

identify the honey bee haplotypes and the evolutionary lineage/-sublineage to which the analysed colonies belonged.

2.4. Data analyses

All analyses were performed at the archipelago level due to the unbalanced sampling among islands. Thus, we analysed temporal trend in pathogen prevalence through a Kruskal-Wallis test excluding data from 2016 and 2017 as we only have one observation per year. We considered three groups of samples according to the date they were collected: 1998, 2008 and 2010–2011. Data of apiaries sampled in Gran Canaria in 1998 were excluded from the analysis because it was an outlier as all sampled colonies tested positive for the pathogen. Furthermore, we tested pathogen prevalence by honey bee haplotype at the archipelago level through a Fisher test. We performed a linear model to test whether the proportion of introduced honey bee haplotypes per island explained pathogen prevalence variation among islands using data from 2008 to 2017. Data from 1998 were excluded because there were only introduced honey bee haplotypes on one island at a very low frequency. Finally, we conducted a Pearson's X^2 test to analyse whether conservation programmes to preserve the genetic integrity of the black Canarian honey bee applied in some islands during different periods had an effect on the pathogen presence detected. We used data from the samplings carried out in Gran Canaria and La Palma in 2016 and 2017 respectively. Conservation measures consisting of a ban on the introduction of foreign queen bees and the breeding and dispersal of Canarian honey queen bees have been applied in La Palma since 2001 (Consejeria de Agricultura, Ganadería, Pesca y Alimentación, 2001) and in Gran Canaria since 2014 (Consejería de Agricultura, Ganadería, 2014). All analyses were carried out in R *vs* 4.1.1 (R Development Core Team, 2021).

3. Results

3.1. Prevalence of trypanosomatids

Trypanosomatids were found in 95 out of 293 analysed samples (32.42 %) distributed across all the sampled islands (Table 1). All these samples were attributed to *L. passim* through BLAST analyses in Genbank while *C. mellificae* was not detected.

Our data showed that *L. passim* was already present in the Canary Islands as early 1998. The prevalence of this pathogen ranged from 16.67 % in 2008 to 60.38 % in 2017, with a marginally significant ($H(2) = 4.911$, $P < 0.086$) trend toward increased prevalence over the study period (Fig. 1).

3.2. Prevalence of neogregarines

Neogregarines were detected in 7 out of the 293 analysed samples (2.38 %). The pathogen was present on all islands, although with a low prevalence. BLAST analyses showed that all the amplicons were identified as *A. bombi*.

Apicystis bombi was found in the sampling from 1998 in Tenerife, and it was also present in samples from 2008, 2010 and 2011 in different islands (Table 1). The pathogen was absent in 2016 and 2017. No statistical analysis was conducted for this pathogen due to its low prevalence.

3.3. Honey bee haplotype and evolutionary lineage/sublineage determination

The analysis of mitochondrial DNA in the 293 sampled colonies revealed the presence of 21 different honey bee haplotypes in the Canary Islands belonging to three lineages (A, C and M) (Table S1 Supplementary data).

Lineage A included 246 samples and the sublineage with Atlantic distribution (A_{III}) was the most frequent (166 colonies), while 35 colonies belonged to the lineage C and 12 were identified as lineage M (Fig. S1 and Table S1 Supplementary data). Data revealed the presence of lineage C in honey bees on Tenerife as early as 1998. The same lineage was found on El Hierro, La Palma y La Gomera in 2008 and was not detected in Gran Canaria until 2010 (Table S1 Supplementary data). The highest percentage of honey bee haplotypes from the lineage C was found in Tenerife, El Hierro and La Gomera. The M lineage was only found in Gran Canaria (2008, 2010 and 2011) where the three lineages coexisted.

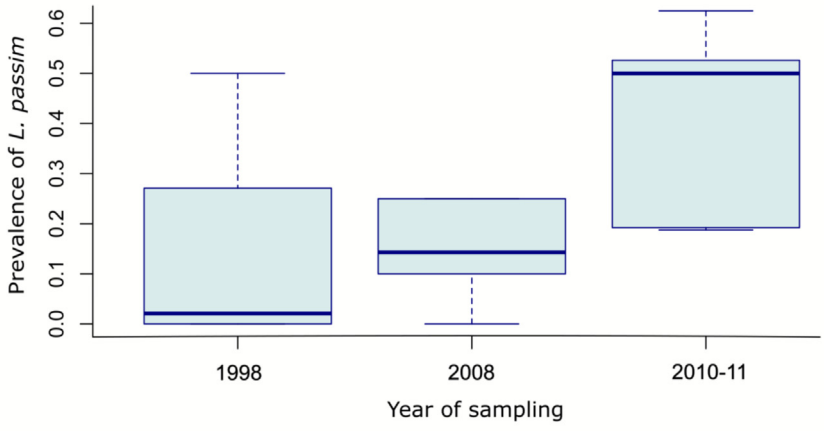

Fig. 1. Prevalence of *L. passim* across the Canarian archipelago along the study period ($P < 0.086$, marginally significant). Colonies sampled in 1998 ($n = 4$ islands), 2008 ($n = 5$ islands) and 2010–2011 ($n = 5$ islands). Boxplots show the median (thick horizontal lines) and the quartiles (boxes and vertical lines). Statistical significance was assessed using $\alpha = 0.05$.

3.4. Correlation of pathogens, honey bee haplotypes and conservation programme

There were no significant differences in *L. passim* prevalence among honey bee haplotypes at the archipelago level (P = 0.224). Interestingly, the proportion of honey bees bearing introduced haplotypes per island did not explain the prevalence of *L. passim* (P = 0.125) (Fig. 2). The duration of the conservation programme was assessed by considering the year of its implementation on each island (2001 in La Palma and 2014 in Gran Canaria) and showed a significant impact on pathogen prevalence (X^2 (1, n = 106) = 19.251, P < 0.001). Notably, the long-standing conservation measures in La Palma were associated with a higher prevalence of *L. passim* (Table 1).

4. Discussion

This study presents the first spatio-temporal analysis of the presence and prevalence of trypanosomatids and neogregarines in *A. mellifera* colonies across the Canary Islands. Our results confirm the presence of *L. passim* in the archipelago as early as 1998, representing the earliest known global record of this pathogen nearly a decade before the previous earliest report of the pathogen worldwide (Castelli et al., 2019; Stevanovic et al., 2016). Furthermore, this pathogen was detected on every island included in the study. *Crithidia mellificae* was not detected and *A. bombi* was found at a very low prevalence. Interestingly, the prevalence of *L. passim* was not associated with the introduction of *A. mellifera* of foreign origin in the Canary Islands, unlike other pathogens such as *N. ceranae* which spread through the islands due to these introductions (Muñoz et al., 2014). Therefore, our results suggest that the emerging pathogen *L. passim* was already present before the introduction of foreign honey bee queens.

Only *L. passim* was detected among trypanosomatids while *C. mellificae* was not found. This aligns with other studies in the field which also found that *L. passim* is the most frequent trypanosomatid species and is a common pathogen in *A. mellifera* (Arismendi et al., 2016; Ravoet et al., 2015; Schwarz et al., 2015a; Stevanovic et al., 2016), while *C. mellificae* prevalence is usually scarce (Buendía et al., 2018; Ravoet et al., 2015) and it has not even been reported in some areas such as the Azores and Madeira (Aguado-López et al., 2023). Indeed, the prevalence of *C. mellificae* was probably overestimated in studies conducted before the description of *L. passim* in 2015 (Schwarz et al., 2015a) since both species are phylogenetically close and sequences from *L. passim* before that date were misidentified as *C. mellificae*. On the other hand, we found that *L. passim* was already present in the archipelago in 1998, which constitutes the earliest report of the species in *A. mellifera* worldwide that was established until now in 2007 in Uruguay (Castelli et al., 2019) and Serbia (Stevanovic et al., 2016). Furthermore, an increasing trend in pathogen prevalence was documented, consistent with findings from related studies in Belgium, Japan, Chile, Serbia and Switzerland (Williams et al., 2019). In the case of the studies conducted by Stevanovic et al. (2016) in Serbia (2007–2015) and by us in the Canary Islands (1998–2017), the lack of samples from previous years makes it impossible to determine when *L. passim* first appeared in these regions. However, investigations conducted in South America by Castelli et al. (2019) included samples from Uruguay (1990–2007), with the earliest detection of the pathogen in 2007, suggesting its absence in earlier years. More studies using historical samples from other regions are needed to clarify whether *L. passim* should be classified as an emerging pathogen or it has long been associated with honey bees before the spread of other pathogens such as *N. ceranae* or *V. destructor*.

Neogregarines were detected at low frequency and all the samples were identified as *A. bombi*. This is consistent with previous studies indicating sporadic presence of the pathogen in *A. mellifera* (Buendía et al., 2018; Morimoto et al., 2013; Plischuk et al., 2011). This pathogen was first reported in *A. mellifera* in 1990 in Finland (Lipa and Triggiani, 1992), and later, in honey bees from other regions (Buendía et al., 2018; Maharramov et al., 2013; Morimoto et al., 2013; Plischuk et al., 2011) thanks to the development of molecular methods (Meeus et al., 2010). Our data contribute another record of *A. bombi* in honey bees, specifically within the Canary Islands. Despite its low prevalence, *A. bombi* was detected on all sampled islands, possibly reflecting spillover from its primary host, bumblebees.

The introduction of foreign honey bee queens to the islands may facilitate the spread of pathogens in confined environments (Muñoz et al., 2014). This study identified 21 honey bee haplotypes, mainly from the lineage A (83.96 %), which is typical of the Macaronesian islands, including the Azores and Madeira (De la Rúa et al., 2006), as well as the Canary Islands (De la Rúa et al., 1998). In contrast, lineages C and M are considered non-native from the Canary Islands, indicating past introductions of honey bees from Europe or even America (De la Rúa et al., 2001; Muñoz et al., 2014). It would be expected to find a correlation

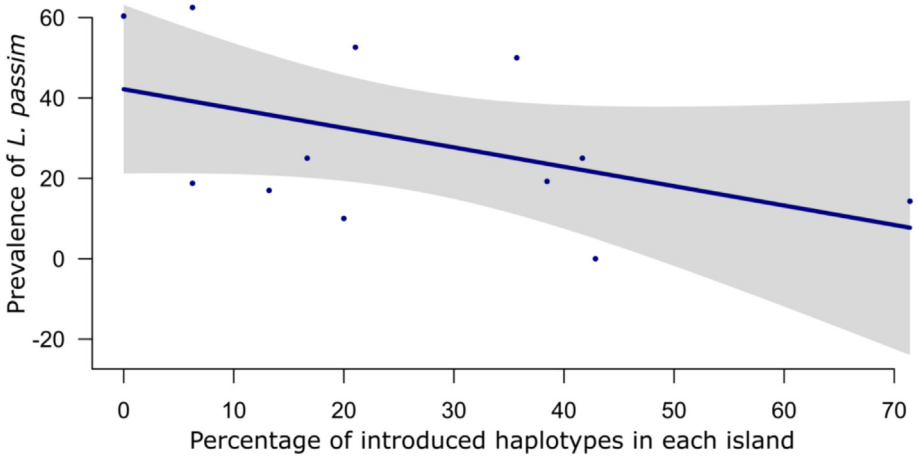

Fig. 2. Relationship between the proportion of introduced honey bee haplotypes in each island and the prevalence of *L. passim* across the Canarian archipelago (each point represents one island; P = 0.125, not significant). The solid line shows the regression fitted to island-level values, and the shaded grey area represents the 95 % confidence interval. Statistical significance was assessed using α = 0.05.

between the introduction of foreign honey bee haplotypes and the spread of trypanosomatids across the islands. However, our analyses revealed no significant association, unlike what has been observed with other pathogens such as *N. ceranae* (Muñoz et al., 2014). Moreover, *L. passim* was already present in 1998 when foreign honey bee haplotypes were absent. This suggests that the pathogen could have arrived in earlier introduction events or might even be endemic to the islands.

Lotmaria passim showed widespread distribution across the archipelago being present on all surveyed islands. Beekeeping practises differ across the Canary Islands: more intensive in Gran Canaria and Tenerife, and more traditional in La Palma, El Hierro and La Gomera (Gobierno de Canarias, 2024). These different management issues may affect pathogen dynamics. For instance, in 2001, a Canarian black honey bee conservation programme was established in La Palma to safeguard the genetic integrity of the local ecotype of honey bees (Consejeria de Agricultura, Ganadería, Pesca y Alimentación, 2001). This strategy involved strict restrictions to the introduction of honey bees into the island as well as special breeding programmes to promote the local ecotype. Previous studies demonstrated that the introduction of foreign honey bee queens triggered the spread of emerging pathogens such as *N. ceranae* through the Canary Islands (Muñoz et al., 2014). Therefore, this special management in La Palma could reduce the prevalence of *L. passim* in the islands. However, these measures seemed not to have any impact on the prevalence of this trypanosomatid in La Palma since our data show that their frequency is similar to the one in other islands from the archipelago. Furthermore, we found that pathogen prevalence in La Palma is higher than in Gran Canaria, where conservation measures were applied much later (in 2014) (Consejería de Agricultura, Ganadería, 2014). Similar findings were reported in the Azores and Madeira where trypanosomatids were even present on islands with strong restrictions on the introduction of honey bees (Aguado-López et al., 2023). Our data show *L. passim* was present in La Palma in 1998, indicating long-term persistence. Thus, conservation measures may be ineffective at limiting *L. passim* prevalence. It would be paramount to conduct further studies to test whether there is any difference in the prevalence of trypanosomatids among islands since our data are scarce to properly address this hypothesis.

There are no reports of colony losses during this period in the Canary Islands, unlike in other areas of Europe and the US where *L. passim* has been associated with honey bee declines (Cepero et al., 2014; Cornman et al., 2012; Ravoet et al., 2013). This may indicate a limited pathogenic role for *L. passim* under local conditions, or the existence of context-dependent interactions with environmental and/or management factors. Moreover, our results suggest that *L. passim* may be endemic to the islands, which could imply the presence of local adaptations in native honey bee populations and/or reduced pathogen virulence. These factors may help explain why the archipelago has not experienced the colony losses reported elsewhere. While genetic and management-related factors may influence pathogen prevalence across islands, the role of agrochemical exposure should not be underestimated, as its interaction with pathogens can substantially increase honey bee mortality (Doublet et al., 2015; Grassl et al., 2018). For instance, exposure to fipronil and thiacloprid has been found to increase mortality in *N. ceranae*-infected honey bees (Vidau et al., 2011). Likewise, Erban et al. (2023) demonstrated that imidacloprid can significantly increase the prevalence and load of *L. passim*, likely by interfering with the bee's immune regulation. Although we did not assess pesticide residues in this study, such factors could help explain some of the variability observed between islands and years.

In conclusion, *L. passim* and *A. bombi* are both present and geographically widespread in the Canary Islands, with *L. passim* being far more prevalent. The detection of *L. passim* in 1998 constitutes the earliest global record and raises questions about its origin and classification as an emerging pathogen. Mechanisms of pathogenicity of *L. passim* and *A. bombi* remain unclear (Buendía-Abad et al., 2022; Plischuk et al., 2011). However, both pathogens can act synergistically with others pathogens increasing honey bee mortality, as seen in coinfections with *N. ceranae* and *L. passim* (Arismendi et al., 2020; MacInnis et al., 2023). These findings underscore the importance of integrating pathogen surveillance with environmental risk assessments, particularly regarding the use of agrochemicals that may exacerbate the impact of pathogens on bee health compromising honey bee survival (Doublet et al., 2015; Erban et al., 2023; Grassl et al., 2018). Therefore, health surveillance in conservation programmes is advised, especially in insular and genetically valuable populations such as those of the Canary Islands.

CRediT authorship contribution statement

Micaela Sánchez-Aroca: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Irene Muñoz:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Pilar De la Rúa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Vicente Martínez-López:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Acknowledgements

We thank two reviewers and the editor for constructive comments, which significantly enhanced the quality of this manuscript. We would like to acknowledge all the beekeepers that collaborate with us sending samples or allowing us to sample their colonies, especially Elías González San Juan and Elizardo Monzón Gil. We thank Paula Acosta De la Rúa for designing the graphical abstract. This research is supported by the Project TD2021-131316B-I00 financed by MCIN/AEI/10.13039/501100011033 and European Union NextGenerationEU/PRTR.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.ijpara.2025.104767.

References

Aguado-López, D., Bartolomé, C., Lopes, A.R., Henriques, D., Segura, S.K., Maside, X., Pinto, M.A., Higes, M., Martín-Hernández, R., 2023. Frequent parasitism of *Apis mellifera* by Trypanosomatids in geographically isolated areas with restricted beekeeping movements. Microb. Ecol. 86, 2655–2665. https://doi.org/10.1007/s00248-023-02266-y.

Arismendi, N., Bruna, A., Zapata, N., Vargas, M., 2016. PCR-specific detection of recently described *Lotmaria passim* (Trypanosomatidae) in Chilean apiaries. J. Invertebr. Pathol. 134, 1–5. https://doi.org/10.1016/j.jip.2015.12.008.

Arismendi, N., Caro, S., Castro, M.P., Vargas, M., Riveros, G., Venegas, T., 2020. Impact of mixed infections of gut parasites *Lotmaria passim* and *Nosema ceranae* on the lifespan and immune-related biomarkers in *Apis mellifera*. Insect 11, 420. https://doi.org/10.3390/insects11070420.

Bojko, J., Becnel, J., Bessette, E., Edwards, S., Gao, J., Huang, W.F., Katanić, N., Khalaf, A., Li, T., Snow, J.W., Solter, L.F., Stratton, C.E., Tokarev, Y., Williams, B., Chen, Y., 2025. Nosema or vairimorpha: genomic/proteomic support to a complex socio-economic issue rooted in taxonomic change. J. Invertebr. Pathol. 212. https://doi.org/10.1016/j.jip.2025.108376.

Bordin, F., Zulian, L., Granato, A., Caldon, M., Colamonico, R., Toson, M., Trevisan, L., Biasion, L., Mutinelli, F., 2022. Presence of known and emerging honey bee pathogens in apiaries of Veneto Region (Northeast of Italy) during Spring 2020 and 2021. Appl. Sci. 12. https://doi.org/10.3390/app12042134.

Buendía-Abad, M., García-Palencia, P., de Pablos, L.M., Alunda, J.M., Osuna, A., Martín-Hernández, R., Higes, M., 2022. First description of *Lotmaria passim* and *Crithidia mellificae* haptomonad stages in the honeybee hindgut. Int. J. Parasitol. 52, 65–75. https://doi.org/10.1016/j.ijpara.2021.06.005.

Buendía, M., Martín-hernández, R., Ornosa, C., Barrios, L., Bartolomé, C., Higes, M., 2018. Epidemiological study of honeybee pathogens in Europe : the results of Castilla-La Mancha (Spain). Spanish J. Agric. Res. 16, 1–10. https://doi.org/10.5424/sjar/2018162-11474.

Castelli, L., Branchiccela, B., Invernizzi, C., Tomasco, I., Basualdo, M., Rodriguez, M., Zunino, P., Antúnez, K., 2019. Detection of *Lotmaria passim* in Africanized and European honey bees from Uruguay, Argentina and Chile. J. Invertebr. Pathol. 160, 95–97. https://doi.org/10.1016/j.jip.2018.11.004.

Cepero, A., Ravoet, J., Gómez-Moracho, T., Bernal, J.L., Del Nozal, M.J., Bartolomé, C., Maside, X., Meana, A., González-Porto, A.V., De Graaf, D.C., Martín-Hernández, R., Higes, M., 2014. Holistic screening of collapsing honey bee colonies in Spain: a case study. BMC. Res. Notes 7. https://doi.org/10.1186/1756-0500-7-649.

Chávez-Galarza, J., Garnery, L., Henriques, D., Neves, C.J., Loucif-Ayad, W., Jonhston, J.S., Pinto, M.A., 2017. Mitochondrial DNA variation of *Apis mellifera iberiensis*: further insights from a large-scale study using sequence data of the tRNAleu-cox2 intergenic region. Apidologie 48, 533–544. https://doi.org/10.1007/s13592-017-0498-2.

Consejería de Agricultura, Ganadería, P.Y.A., 2001. Orden de 6 abril de 2001, por la que se establecen medidas especiales de protección para la conservación, recuperación y selección de la Abeja Negra Canaria. Bol. Of. Canar. 49.

Consejería de Agricultura, Ganadería, P.Y.A., 2014. ORDEN de 23 de abril de 2014, por la que se extienden a la isla de Gran Canaria las medidas especiales de protección para la conservación, recuperación y selección de la Abeja Negra Canaria establecidas en la Orden de 6 de abril de 2001, de la Consejería. Boletín Of. Canar. 86.

Cornman, R.S., Tarpy, D.R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J.S., VanEngelsdorp, D., Evans, J.D., 2012. Pathogen webs in collapsing honey bee colonies. PLoS One 7, e43562. https://doi.org/10.1093/genetics/128.2.393.

Cornuet, J.-M., Garnery, L., Solignac, M., 1991. Putative origin and function of the intergenic region between COI and COII of *Apis mellifera* L. mitochondrial DNA. Genetics 128, 393–403. https://doi.org/10.1093/genetics/128.2.393.

De la Rúa, P., Galián, J., Pedersen, B.V., Serrano, J., 2006. Molecular characterization and population structure of *Apis mellifera* from Madeira and the Azores. Apidologie 37, 699–708. https://doi.org/10.1051/apido:2006044.

De la Rúa, P., Galián, J., Serrano, J., Moritz, R.F.A., 2001. Genetic structure and distinctness of *Apis mellifera* L. populations from the Canary Islands. Mol. Ecol. 10, 1733–1742. https://doi.org/10.1046/j.1365-294X.2001.01303.x.

De la Rúa, P., Jaffé, R., Dall'Olio, R., Muñoz, I., Serrano, J., 2009. Biodiversity, conservation and current threats to European honeybees. Apidologie 40, 263–284. https://doi.org/10.1051/apido/2009027.

De la Rúa, P., Serrano, J., Galián, J., 1998. Mitochondrial DNA variability in the Canary Islands honeybees (*Apis mellifera* L.). Mol. Ecol. 7, 1543–1547. https://doi.org/10.1046/j.1365-294x.1998.00468.x.

De la Rúa, P., Serrano, J., Galián, J., 2002. Biodiversity of *Apis mellifera* populations from Tenerife (Canary Islands) and hybridisation with East European races. Biodivers. Conserv. 11, 59–67. https://doi.org/10.1023/A:1014066407307.

de Miranda, J., Meeus, I., Yañez, O., Piot, N., Jara, L., Onorati, P., la Rúa, P.D., Nielsen, A., Tlak-Gajger, I., Özgör, E., Dalmon, A., Gajda, A., Neumann, P., Smagghe, G., Paxton, R., 2021. Protocols for assessing the distribution of pathogens in individual Hymenopteran pollinators. Res. Sq. https://doi.org/10.21203/rs.3.pex-1453/v1.

Delaplane, K.S., Mayer, D.F., 2000. Crop Pollination by Bees. CABI Publishing, Wallingford, UK and New York, USA.

Doublet, V., Labarussias, M., de Miranda, J.R., Moritz, R.F.A., Paxton, R.J., 2015. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environ. Microbiol. 17, 969–983. https://doi.org/10.1111/1462-2920.12426.

Ellis, J.D., Munn, P.A., 2005. The worldwide health status of honey bees. Bee World 86, 88–101. https://doi.org/10.1080/0005772X.2005.11417323.

Erban, T., Parizkova, K., Sopko, B., Talacko, P., Markovic, M., Jarosova, J., Votypka, J., 2023. Imidacloprid increases the prevalence of the intestinal parasite *Lotmaria passim* in honey bee workers. Sci. Total Environ. 905, 166973. https://doi.org/10.1016/j.scitotenv.2023.166973.

Evans, J.D., Schwarz, R.S., Chen, Y.P., Budge, G., Cornman, R.S., De la Rua, P., de Miranda, J.R., Foret, S., Foster, L., Gauthier, L., 2013. Standard methods for molecular research in *Apis mellifera*. J. Apic. Res. 52, 1–54. https://doi.org/10.3896/IBRA.1.52.4.11.

Figueroa, L.L., Compton, S., Grab, H., McArt, S.H., 2021. Functional traits linked to pathogen prevalence in wild bee communities. Sci. Rep. 11, 1–12. https://doi.org/10.1038/s41598-021-87103-3.

Gallai, N., Salles, J.M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68, 810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014.

Garnery, L., Solignac, M., Celebrano, G., Cornuet, J.-M., 1993. A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera* L. Experientia 49, 1016–1021. https://doi.org/10.1007/BF02125651.

Gobierno de Canarias, 2024. Instituto Canario de Estadística (ISTAC) [WWW Document]. https://www3.gobiernodecanarias.org/istac/statistical-visualizer/visualizer/collection.html?resourceType=collection&agencyId=ISTAC&resourceId=E01008B_000001.

Gómez-Moracho, T., Buendía-abad, M., Benito, M., García-palencia, P., Barrios, L., Bartolomé, C., Maside, X., Meana, A., Jiménez-antón, M.D., Olías-molero, A.I., María, J., Martín-hernández, R., Higes, M., 2020. Experimental evidence of harmful effects of *Crithidia mellificae* and *Lotmaria passim* on honey bees. Int. J. Parasitol. 50 (13), 1117–1124. https://doi.org/10.1016/j.ijpara.2020.06.009.

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science (80-.) 347, 1255957. https://doi.org/10.1126/science.1255957.

Grassl, J., Holt, S., Cremen, N., Peso, M., Hahne, D., Baer, B., 2018. Synergistic effects of pathogen and pesticide exposure on honey bee (*Apis mellifera*) survival and immunity. J. Invertebr. Pathol. 159, 78–86. https://doi.org/10.1016/j.jip.2018.10.005.

Grupe, A.C., Quandt, A., 2020. A growing pandemic: a review of *Nosema* parasites in globally distributed domesticated and native bees. PLoS Pathog. 16, 1–8. https://doi.org/10.1371/journal.ppat.1008580.

Hubert, J., Bicianova, M., Ledvinka, O., Kamler, M., Lester, P.J., Nesvorna, M., Kopecky, J., Erban, T., 2017. Changes in the bacteriome of honey bees associated with the parasite *Varroa destructor*, and pathogens *Nosema* and *Lotmaria passim*. Microb. Ecol. 73, 685–698. https://doi.org/10.1007/s00248-016-0869-7.

Ivanova, N., Dewaard, J., Hebert, P., 2006. An inexpensive automatio-friendly protocol for recovering high-quality DNA. Mol. Ecol. https://doi.org/10.1111/j.1471-8286.2006.01428.x.

Jaffé, R., Dietemann, V., Allsopp, M.H., Costa, C., Crewe, R.M., Dall'Olio, R., De la Rúa, P., El-Niweiri, M.A.A., Fries, I., Kezic, N., Meusel, M.S., Paxton, R.J., Shaibi, T., Stolle, E., Moritz, R.F.A., 2010. Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. Conserv. Biol. 24, 583–593. https://doi.org/10.1111/j.1523-1739.2009.01331.x.

Jara, L., Ruiz, C., Martín-Hernández, R., Muñoz, I., Higes, M., Serrano, J., De la Rúa, P., 2020. The effect of migratory beekeeping on the infestation rate of parasites in honey bee (*Apis mellifera*) colonies and on their genetic variability. Microorganisms 9, 22. https://doi.org/10.3390/microorganisms9010022.

Jones, C.M., Brown, M.J.F., 2014. Parasites and genetic diversity in an invasive bumblebee. J. Anim. Ecol. 83, 1428–1440. https://doi.org/10.1111/1365-2656.12235.

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/rspb.2006.3721.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Langridge, D.F., McGhee, R.B., 1967. *Crithidia mellificae* n. sp. an acidophilic trypanosomatid of the honey bee *Apis mellifera*. J. Protozool. 14, 485–487. https://doi.org/10.1111/j.1550-7408.1967.tb02033.x.

Lee, K.V., Steinhauer, N., Rennich, K., Wilson, M.E., Tarpy, D.R., Caron, D.M., Rose, R., Delaplane, K.S., Baylis, K., Lengerich, E.J., Pettis, J., Skinner, J.A., Wilkes, J.T., Sagili, R., vanEngelsdorp, D., Partnership for the B.I., 2015. A national survey of managed honey bee 2013–2014 annual colony losses in the USA. Apidologie 46, 292–305. https://doi.org/10.1007/s13592-015-0356-z.

Lipa, J.J., Triggiani, O., 1996. *Apicystis* gen nov and *Apicystis bombi* (Liu, Macfarlane & Pengelly) comb nov (Protozoa: Neogregarinida), a cosmopolitan parasite of *Bombus* and *Apis* (Hymenoptera: Apidae). Apidologie 27, 29–34. https://doi.org/10.1051/apido:19960104.

Lipa, J.J., Triggiani, O., 1992. A newly recorded neogregarine (Protozoa, Apicomplexa), parasite in honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp). Apidologie 23, 533–536. https://doi.org/10.1051/apido:19920605.

Liu, Q., Lei, J., Darby, A.C., Kadowaki, T., 2020. Trypanosomatid parasite dynamically changes the transcriptome during infection and modifies honey bee physiology. Commun. Biol., 1–8 https://doi.org/10.1038/s42003-020-0775-x.

Macfarlane, R.P., Lipa, J.J., Liu, H.J., 1995. Bumble bee pathogens and internal enemies. Bee World 76, 130–148. https://doi.org/10.1080/0005772X.1995.11099259.

MacInnis, C.I., Luong, L.T., Pernal, S.F., 2025. Effects of *Nosema ceranae* and *Lotmaria passim* infections on honey bee foraging behaviour and physiology. Int. J. Parasitol. 55, 213–223. https://doi.org/10.1016/j.ijpara.2024.12.003.

MacInnis, C.I., Luong, L.T., Pernal, S.F., 2023. A tale of two parasites : responses of honey bees infected with *Nosema ceranae* and *Lotmaria passim*. Sci. Rep., 1–11 https://doi.org/10.1038/s41598-023-49189-9.

Maharramov, J., Meeus, I., Maebe, K., Arbetman, M., Morales, C., Graystock, P., Hughes, W.O.H., Plischuk, S., Lange, C.E., De Graaf, D.C., 2013. Genetic variability of the neogregarine *Apicystis bombi*, an etiological agent of an emergent bumblebee disease. PLoS One 8, e81475. https://doi.org/10.1371/journal.pone.0081475.

Martín-Hernández, R., Bartolomé, C., Chejanovsky, N., Le Conte, Y., Dalmon, A., Dussaubat, C., García-Palencia, P., Meana, A., Pinto, M.A., Soroker, V., Higes, M., 2018. *Nosema ceranae* in *Apis mellifera*: a 12 years postdetection perspective. Environ. Microbiol. 20, 1302–1329. https://doi.org/10.1111/1462-2920.14103.

Martínez-López, V., Ruiz, C., De la Rúa, P., 2022a. Migratory beekeeping and its influence on the prevalence and dispersal of pathogens to managed and wild bees. Int. J. Parasitol. Parasites Wildl. 18, 184–193. https://doi.org/10.1016/j.ijppaw.2022.05.004.

Martínez-López, V., Ruiz, C., Muñoz, I., Ornosa, C., Higes, M., Martín-Hernández, R., De la Rúa, P., 2022b. Detection of microsporidia in pollinator communities of a Mediterranean biodiversity hotspot for wild bees. Microb. Ecol. 84, 638–642. https://doi.org/10.1007/s00248-021-01854-0.

Meana, A., Llorens-Picher, M., Euba, A., Bernal, J.L., Bernal, J., García-Chao, M., Dagnac, T., Castro-Hermida, J.A., Gonzalez-Porto, A.V., Higes, M., 2017. Risk factors associated with honey bee colony loss in apiaries in Galicia, NW Spain. Spanish J. Agric. Res. 15, e0501. https://doi.org/10.5424/sjar/2017151-9652.

Meeus, I., De Graaf, D.C., Jans, K., Smagghe, G., 2010. Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers. J. Appl. Microbiol. 109, 107–115. https://doi.org/10.1111/j.1365-2672.2009.04635.x.

Meixner, M.D., Maria Alice, P., Maria, B., Per, K., Evgeniya, I., Fuchs, S., 2013. Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. J. Apic. Res. 52, 1–28. https://doi.org/10.3896/IBRA.1.52.4.05.

Meusel, M.S., Moritz, R.F.A., 1993. Transfer of paternal mitochondrial DNA during fertilization of honeybee (*Apis mellifera* L.) eggs. Curr. Genet. 24, 539–543. https://doi.org/10.1007/BF00351719.

Miguel, I., Lionel, G., Mikel, I., Michel, B., Carmen, M., W, S.S., Estonba, A., 2015. Origin, evolution and conservation of the honey bees from La Palma Island (Canary Islands): molecular and morphological data. J. Apic. Res. 54, 427–440. https://doi.org/10.1080/00218839.2016.1180017.

Morimoto, T., Kojima, Y., Yoshiyama, M., Kimura, K., Yang, B., Peng, G., Kadowaki, T., 2013. Molecular detection of protozoan parasites infecting *Apis mellifera* colonies in Japan. Environ. Microbiol. Rep. 5, 74–77. https://doi.org/10.1111/j.1758-2229.2012.00385.x.

Mráz, P., Hýbl, M., Kopecký, M., Bohatá, A., Hoštičková, I., Šipoš, J., Vočadlová, K., Čurn, V., 2021. Screening of honey bee pathogens in the czech republic and their prevalence in various habitats. Insects 12. https://doi.org/10.3390/insects12121051.

Muñoz, I., Cepero, A., Pinto, M.A., Martín-Hernández, R., Higes, M., De la Rúa, P., 2014. Presence of *Nosema ceranae* associated with honeybee queen introductions. Infect. Genet. Evol. 23, 161–168. https://doi.org/10.1016/j.meegid.2014.02.008.

Muñoz, I., De la Rúa, P., 2012. Temporal analysis of the genetic diversity in a honey bee mating area of an island population (La Palma, Canary Islands, Spain). J. Apic. Sci. 56, 41–49. https://doi.org/10.3896/IBRA.1.51.1.20.

Muñoz, I., Pinto, M.A., De la Rúa, P., 2013. Temporal changes in mitochondrial diversity highlights contrasting population events in Macaronesian honey bees. Apidologie 44, 295–305. https://doi.org/10.1007/s13592-012-0179-0.

Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x.

Pinto, M.A., Henriques, D., Neto, M., Guedes, H., Muñoz, I., Azevedo, J., De la Rúa, P., 2013. Maternal diversity patterns of Ibero-Atlantic populations reveal further complexity of Iberian honeybees. Apidologie 44, 430–439. https://doi.org/10.1007/s13592-013-0192-y.

Pinto, M.A., Muñoz, I., Chávez-Alarza, J., De la Rúa, P., 2012. The Atlantic side of the Iberian Peninsula: a hot-spot of novel African honey bee maternal diversity. Apidologie 43, 663–673. https://doi.org/10.1007/s13592-012-0141-1.

Plischuk, S., Meeus, I., Smagghe, G., Lange, C.E., 2011. *Apicystis bombi* (Apicomplexa: Neogregarinorida) parasitizing *Apis mellifera* and *Bombus terrestris* (Hymenoptera: Apidae) in Argentina. Environ. Microbiol. Rep. 3, 565–568. https://doi.org/10.1111/j.1758-2229.2011.00261.x.

R Development Core Team, 2021. R: A Language An Environment for Statistical Computing.

Ratnieks, F.L.W., Carreck, N.L., 2010. Ecology. Clarity on honey bee collapse? Science 327, 152–153. https://doi.org/10.1126/science.1185563.

Ravoet, J., Maharramov, J., Meeus, I., De Smet, L., Wenseleers, T., Smagghe, G., de Graaf, D.C., 2013. Comprehensive bee pathogen screening in Belgium reveals *Crithidia mellificae* as a new contributory factor to winter mortality. PLoS One 8, e72443. https://doi.org/10.1371/journal.pone.0072443.

Ravoet, J., Schwarz, R.S., Descamps, T., Yañez, O., Tozkar, C.O., Martin-Hernandez, R., Bartolomé, C., De Smet, L., Higes, M., Wenseleers, T., Schmid-Hempel, R., Neumann, P., Kadowaki, T., Evans, J.D., de Graaf, D.C., 2015. Differential diagnosis of the honey bee trypanosomatids *Crithidia mellificae* and *Lotmaria passim*. J. Invertebr. Pathol. 130, 21–27. https://doi.org/10.1016/j.jip.2015.06.007.

Ribani, A., Utzeri, V.J., Taurisano, V., Galuppi, R., Fontanesi, L., 2021. Analysis of honey environmental DNA indicates that the honey bee (*Apis mellifera* L.) trypanosome parasite *Lotmaria passim* is widespread in the apiaries of the North of Italy. J. Invertebr. Pathol. 184, 107628. https://doi.org/10.1016/j.jip.2021.107628.

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103 (Suppl), S96–S. https://doi.org/10.1016/j.jip.2009.07.016.

Rutrecht, S.T., Brown, M.J.F., 2008. The life-history impact and implications of multiple parasites for bumble bee queens. Int. J. Parasitol. 38, 799–808. https://doi.org/10.1016/j.ijpara.2007.11.004.

Schmid-Hempel, P., 2001. On the evolutionary ecology of host–parasite interactions: addressing the question with regard to bumblebees and their parasites. Naturwissenschaften 88, 147–158. https://doi.org/10.1007/s001140100222.

Schwarz, R.S., Bauchan, G.R., Murphy, C.A., Ravoet, J., de Graaf, D.C., Evans, J.D., 2015a. Characterization of two species of trypanosomatidae from the honey bee *Apis mellifera*: *Crithidia mellificae* Langridge and McGhee, and *Lotmaria passim* n. gen., n. sp. J. Eukaryot. Microbiol. 62, 567–583. https://doi.org/10.1111/jeu.12209.

Schwarz, R.S., Huang, Q., Evans, J.D., 2015b. Hologenome theory and the honey bee pathosphere. Curr. Opin. Insect Sci. 10, 1–7. https://doi.org/10.1016/j.cois.2015.04.006.

Stevanovic, J., Schwarz, R.S., Vejnovic, B., Evans, J.D., Irwin, R.E., Glavinic, U., Stanimirovic, Z., 2016. Species-specific diagnostics of *Apis mellifera* trypanosomatids: a nine-year survey (2007–2015) for trypanosomatids and microsporidians in Serbian honey bees. J. Invertebr. Pathol. 139, 6–11. https://doi.org/10.1016/j.jip.2016.07.001.

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J.L., Texier, C., Biron, D.G., Blot, N., Alaoui, H., Belzunces, L.P., Delbac, F., 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS One 6. https://doi.org/10.1371/journal.pone.0021550.

Williams, M.-K.-F., Tripodi, A.D., Szalanski, A.L., 2019. Molecular survey for the honey bee (*Apis mellifera* L.) trypanosome parasites *Crithidia mellificae* and *Lotmaria passim*. J. Apic. Res. 58, 553–558. https://doi.org/10.1080/00218839.2019.1568956.