



Culture-based survey of fungi from decaying *Vespa orientalis* colonies reveals *Beauveria varroae* as a virulent and horizontally transmissible entomopathogen

Ernesto Ragusa^a, Karen Power^{b,*}, Livio Torta^a, Marika Lamendola^a, Giovanni Jesu^c, Giovanna De Leva^c, Alessandro Scavuzzo^a, Laura Bortolotti^d, Andrea Becchimanzi^{c,e,**}

^a Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy

^b Department of Biology, University of Naples "Federico II", Naples, Italy

^c Department of Agricultural Sciences, University of Naples "Federico II", Naples, Italy

^d Council for agricultural research and economics, Research Centre for Agriculture and Environment, Bologna, Italy

^e BAT Center - Interuniversity Center for Studies On Bioinspired Agro-Environmental Technology, University of Naples "Federico II", Naples, Italy

ARTICLE INFO

Keywords:

Hornet biocontrol
Vespidae histopathology
Entomopathogens
Colony-associated fungi
Mycobiota

ABSTRACT

The oriental hornet (*Vespa orientalis*) is increasingly recognized as a significant pest of honey bees in southern Europe and other dry sub-humid regions, where it causes colony losses, disrupts foraging activity, and may contribute to pathogen circulation among apiaries. Current control measures, based mainly on trapping and nest destruction, are labor-intensive and raise concerns for non-target organisms, highlighting the need for sustainable alternatives. Entomopathogenic fungi represent promising candidates due to their insecticidal activity and potential for horizontal transmission within social groups. Here we characterized the fungal community associated with decaying *V. orientalis* nests and identified *Beauveria varroae* as a naturally occurring entomopathogen. The nest-associated strain was compared with a commercial *Beauveria bassiana* strain (ATCC 74040; Naturalis®) in laboratory bioassays on adult hornets using contact, ingestion, and indirect transmission routes. Survival analyses revealed significant route-dependent mortality for both strains. Histological observations confirmed fungal penetration and systemic colonization, particularly of fat body tissues, and indicated host immune responses at lower inoculum levels. Indirect transmission assays demonstrated the capacity of infected individuals to disseminate conidia to conspecifics. These findings identify *B. varroae* as a virulent pathogen of *V. orientalis* and support the feasibility of exploiting nest-level horizontal transmission ("Trojan horse" strategy) for hornet management. Further research should focus on optimizing delivery systems and assessing field applicability within integrated pest management frameworks.

1. Introduction

Hornets of the genus *Vespa* (Hymenoptera: Vespidae) are among the most effective predators of honey bees, exerting strong pressure on colonies through direct predation and persistent foraging interference (Abd Al-Fattah et al., 2014; Matsuura et al., 1990; Tan et al., 2013). Most *Vespa* species are native to East and Southeast Asia (Richards, 1971), but several aspects of their biology facilitate their successful invasion of habitats in nonnative regions (Otis et al., 2023), where they often establish becoming invasive pests (Arca et al., 2015; Kim et al., 2006; Pusceddu et al., 2022; Sánchez et al., 2024; Werenkraut et al., 2022;

Wilson et al., 2020). The impact of *Vespa* spp. invasions are not limited to managed honey bee populations, but may extend to native arthropod communities through predation, competition and fear (Otis et al., 2023; Tan et al., 2013), with potential negative effects on pollination and natural biocontrol (Beggs et al., 2011; Hanna et al., 2014). Even *V. crabro* and *V. orientalis*, which are naturally distributed across Europe and the Mediterranean basin (Cetkovic, 2003), may behave as pests when introduced in novel subregional areas (Pusceddu et al., 2022; Temreshev, 2018; Zucca et al., 2024).

Over the past decade, partly due to anthropogenic transport and climatic suitability, *V. orientalis* has expanded its range beyond its

* Corresponding author.

** Corresponding author at: Department of Agricultural Sciences, University of Napoli "Federico II", Napoli, Italy.

E-mail addresses: karen.power@unina.it (K. Power), andrea.becchimanzi@unina.it (A. Becchimanzi).

<https://doi.org/10.1016/j.jip.2026.108728>

Received 28 March 2026; Received in revised form 21 August 2026; Accepted 27 August 2026

Available online 28 August 2026

0022-2011/© 2026 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

traditional Mediterranean distribution and has been recorded in several European countries, including Spain (Hernández et al., 1999), Romania (Zachi and Ruicănescu, 2021), France (Gereys et al., 2021), and the Greek islands (Ceccolini, 2022). In Italy, the species was historically confined to Sicily, Calabria, Campania, and Lazio (Cetkovic, 2003), but has recently spread to Marche, Tuscany, Sardinia, Liguria, and Friuli Venezia Giulia, where it occurs at variable population densities (Bressi et al., 2019; Graziani and Cianferoni, 2021; Ruschioni et al., 2023). Human-mediated introductions have also been documented in Chile and Mexico (Dvořák, 2006; Ríos V. et al., 2020). In temperate regions, *V. orientalis* exhibits an annual colony cycle (Ishay, 1964). After overwintering, founding queens establish primary nests in early spring, which initially contain a limited number of brood cells (Darchen, 1964). The emergence of the first workers by early summer allows the colony to grow rapidly, reaching peak size during the summer months, with populations of up to 400 individuals (Archer, 1998). Reproductive individuals are produced towards late summer and autumn; newly emerged queens mate and subsequently overwinter before founding new colonies the following year (Ishay, 1964; Matsuura et al., 1990). In dry sub-humid climatic areas such as South Europe, North Africa, West/South Asia, *V. orientalis* is considered one of the most damaging pests of *Apis mellifera*, causing substantial colony losses (Ishay, 1964; Sweelam et al., 2019). Adult hornets primarily consume carbohydrates, such as nectar, honeydew, and ripe fruits (Archer, 1998), whereas larvae require large quantities of protein. Consequently, workers actively prey on large insects, particularly honey bees, and also exploit scavenging resources such as meat and fish (Ishay and Ikan, 1968). Predation pressure on apiaries is greatest between July and November, coinciding with the peak protein demand of developing larvae and maximum colony growth (Archer, 1998; Baracchi et al., 2010). Beyond direct predation, *V. orientalis* represents a potential epidemiological threat to honey bees, contributing to pathogen dissemination among apiaries (Zucca et al., 2024). A recent biomolecular survey in Italy detected six honey bee viruses (ABPV, BQCV, CBPV, DWV, KBV, and SBV), tripanosomatids and *Vairimorpha ceranae* in hornet specimens, with frequent co-infections observed (Power et al., 2024, 2023, 2022). Finally, *V. orientalis* also poses a public health concern: although not more aggressive than other hornets such as *V. crabro*, its tendency to nest and forage in densely populated urban areas increases the likelihood of human-hornet interactions (Smith-Pardo et al., 2024; Zucca et al., 2024).

Current control strategies against hornets rely largely on mechanical trapping, nest destruction, and, in some cases, on synthetic insecticides delivered either by direct nest treatment or via contaminated individuals used to transfer the active substance to the colony. These approaches suffer from major limitations, including low selectivity, variable efficacy, labor intensity, and potential negative effects on non-target insects, including pollinators (Lester, 2025; Malaquias Souto et al., 2025; Rojas-Nossa et al., 2024). Moreover, chemical control is particularly problematic in the context of apiaries, where the risk of affecting bees and contaminating hive products is unacceptable. As a result, there is a growing interest in alternative, environmentally compatible control strategies that can be integrated into sustainable pest management programs (Turchi and Derijard, 2018).

Among biological control options, entomopathogenic fungi (EPF) represent a promising but still underexplored tool for hornet management (Bava et al., 2022; Poidatz et al., 2018). Fungi belonging to the genus *Beauveria* are well known for their broad EPF potential and have been widely studied and applied against agricultural pests. Their pathogenicity relies on a combination of mechanical penetration and enzymatic degradation of the insect cuticle, mediated by proteases, lipases, esterases, and chitinases, followed by colonization of the hemocoel and the production of toxic secondary metabolites, such as beauvericin (Mannino et al., 2019; Pedrini, 2022; Valero-Jiménez et al., 2016b). The infection process typically culminates in host death and the emergence of fungal reproductive structures on the mummified insect cadaver

(Hajek and St. Leger, 1994). Importantly, the quantity and composition of virulence factors and secondary metabolites vary markedly among *Beauveria* species and even among strains of the same species, influencing host specificity and efficacy (Fuguet and Vey, 2004; Quesada-Moraga and Vey, 2003; Ranesi et al., 2024; Valero-Jiménez et al., 2016a). Beyond direct pathogenicity, an important advantage of EPF lies in their potential to be disseminated through insect behavior, enabling indirect transmission within social groups. In social wasps, including *Vespa* spp., workers engage in frequent physical interactions such as grooming, trophallaxis, and nestmate contact, which can facilitate the horizontal transfer of fungal propagules from treated individuals to conspecifics (Hunt et al., 1982; Ishay and Ikan, 1968; Poidatz et al., 2022). This concept has been described as a “Trojan horse” strategy, whereby foraging adults acquire the pathogen outside the nest and subsequently introduce it into the colony environment (Poidatz et al., 2019, 2018). In this context, exploiting nest-associated or host-adapted EPF may further enhance the effectiveness of Trojan horse strategies by improving pathogen persistence, transmission efficiency, and host specificity (Brodeur, 2012). However, the success of such approaches depends on a detailed understanding of fungal virulence, transmission dynamics, and host immune responses, factors that remain largely unexplored for *V. orientalis*.

In this study, we investigated the interaction between *V. orientalis* and *Beauveria* spp. by characterizing the mycobiota associated with hornet nests and isolating *B. varroae* directly from nest material. We then compared its virulence with that of a commercial *B. bassiana* strain (Naturalis®) using different inoculation routes under controlled laboratory conditions. By combining survival analysis, transmission assays, and histopathological observations, our aim was to assess fungal pathogenicity, host responses, and transmission potential, providing a first evaluation of *Beauveria*-based strategies for the biological control of *V. orientalis*.

2. Materials and methods

2.1. Fungal isolation and identification

Fungal isolates were obtained from two collapsing *V. orientalis* colonies collected during 2023 and 2024, in Portici (Naples, Southern Italy) and Palermo (Southern Italy), respectively. Dead individuals and debris were extracted from the nest using sterile forceps and washed in PBS + 0.5% Tween 20. Insects and debris were processed by serial dilution method using sterile distilled water. An aliquot (100 µL) of the suspensions were plated on PDA Petri dishes (9 cm). Washing buffer aliquots (100 µL) were plated as well, to discriminate between inner and outside fungal presence. Fungal colonies were subcultured and identified by molecular techniques targeting ITS regions. DNA extraction with CTAB buffer and PCR using ITS-1F and ITS-4 primers were performed following protocols described elsewhere (Cubero et al., 1999). After sequencing, the isolates were identified by blastn searches against ITS TYPE material db (NCBI). One isolate was identified as *B. varroae* (PDA11Vo712), while a commercial strain of *B. bassiana* (ATCC 74040) was isolated from a biopesticide formulation (Naturalis®, CBC S.r.l., Grassobbio, Italy), by plating 1:100 dilution of the product on PDA.

Phylogenetic analysis was conducted on PDA11Vo712 strain by aligning ITS and RPB1 sequences of isolates from GenBank (Supplementary Table S1), selected among the most similar hits reported by blastn searches against nr/nt and TYPE material databases (NCBI). PCR to amplify a fragment of RPB1 locus, which encodes largest subunit of RNA polymerase II, was performed using the primers RPB1-313F (5'-YTGGARATTGTCTGCCAAY-3') and RPB1CrW (5'-CCNGCDATNTCRTRTCCATRW-3'), following a protocol described elsewhere (Simmons et al., 2015).

ITS and RPB1 sequences were aligned independently using MUSCLE v5.3 (Edgar, 2022) and the resulting alignments were visually inspected and manually curated where necessary using AliView (Larsson, 2014).

Phylogenetic relationships were subsequently investigated using both single-locus and concatenated datasets (ITS = 1–587 bp; RPB1 = 588–1319 bp). For the concatenated analyses, two datasets were generated: (i) a complete two-locus dataset comprising the 14 taxa for which both ITS and RPB1 sequences were available, and (ii) a broader dataset comprising all 24 taxa included in the ITS analysis, with the RPB1 partition coded as missing data for taxa lacking RPB1 sequences. ITS and RPB1 alignments were concatenated while retaining the two loci as separate partitions.

The best-fitting nucleotide substitution model for each locus was selected using ModelTest-NG v0.1.7 (Darrriba et al., 2020) according to the Bayesian Information Criterion (BIC). TIM2 + I + G4 and TrNef+G4 were selected for the ITS and RPB1 partitions, respectively. Maximum-likelihood phylogenetic inference was performed using RAXML-NG v2.0.2 (Kozlov et al., 2019), allowing each partition to evolve according to its respective substitution model. Branch support was assessed using 1000 non-parametric bootstrap replicates. The complete 14-taxon concatenated dataset was used for the primary phylogenetic reconstruction, while the 24-taxon sparse-matrix analysis and the individual ITS and RPB1 phylogenies were used to evaluate the robustness of the inferred taxonomic placement. The phylogenetic tree was drawn using the software FigTree v1.4.3.

2.2. Virulence assay on *V. orientalis* adults

Beauveria species were grown on PDA plates at 25 °C in dark conditions. Conidia were harvested from 10-day-old PDA cultures, scraped from plates, and suspended in water to a final concentration of 10^7 conidia/mL, to perform oral inoculation assays. For direct contact and indirect transmission inoculation assays, conidia were suspended in an emulsion of 5% sunflower oil, 5% Tween 20 and 90% sterile water, to facilitate adhesion to the hornet integument (Ranesi et al., 2024).

Adult females of *V. orientalis* were collected using an entomological net, while attacking managed colonies of *Apis mellifera*. Subsequently, the individuals were transferred into 50-mL Falcon tubes and introduced into observation arenas. Each arena consisted of transparent plastic boxes (16 cm × 11 cm × 6 cm) to which feeding dispensers were attached to provide food to the hornets during the experiments (Supplementary Fig. S4).

All experiments were conducted at the Apidology laboratory of the SAAF Department, University of Palermo, under controlled temperature (25 ± 1 °C) and relative humidity ($70 \pm 5\%$) conditions; the light–dark cycle was 16:8 h. Each trial began after keeping the hornets inside the arenas for 24 h under optimal conditions and adequately fed, so that they could acclimate.

Five adults were placed in each arena, maintained at the above conditions and inoculated with the *B. bassiana* ATCC 74040, *B. varroae* PDA11Vo712, or control solution as described below. After a 24 h acclimation period inside the arenas, hornets were artificially infected using three methods: direct contact, ingestion, and indirect transmission. For each fungal strain and each inoculation method, four arenas (5 hornets per arena) were set up per experimental run. The entire experiment was repeated twice. Each fungal treatment consisted of 40 hornets per inoculation method (5 hornets × 4 arenas × 2 experimental repetitions), whereas the corresponding control treatment consisted of 20 hornets.

To test virulence by direct contact exposure, each hornet was removed from the Falcon tube and restrained using soft plastic laboratory tweezers to avoid injury; then, using a micropipette, a 10 µL drop of conidial suspension, or tween/oil control emulsion without conidia, was applied to the dorsal thorax. After waiting for the emulsion to dry, the hornets were transferred again inside the observation arenas, in groups of five.

To test virulence by ingestion, hornets were fed through the dispensers with a 25% (w/v) sucrose syrup containing 10^7 conidia/mL, for 24 h. Control hornets received the same sucrose solution without

conidia. After the exposure period, the inoculated diet was replaced with 50% (w/v) sucrose syrup, which was provided for the remainder of the experiment.

Finally, to test virulence of *Beauveria* isolates by indirect transmission, one hornet was marked using Uni POSCA Paint Markers (Uni Mitsubishi Pencil, UK) and inoculated by contact, as described above. The topically inoculated hornet was introduced into the arena containing four untreated individuals, allowing secondary exposure through contact. For the corresponding control treatment, the marked carrier hornet was treated identically with the conidial carrier (tween/oil emulsion) but without conidia before being introduced into an arena with four untreated hornets. Two hours after inoculation, observation arenas were checked to replace any individuals that had died from causes not directly attributable to the fungi. Survival was monitored every 24 h for 10 days, removing dead hornets from the arena. Kaplan-Meier survival curves were generated and compared using log-rank tests.

2.3. Macroscopic and microscopic examination

After 10 days, for each experimental condition, surviving individuals were 10% neutral buffered formalin (NFB) fixed and shipped to the Histopathology and Diagnostic Core of the Department of Biology, University of Naples “Federico II”. All samples were observed at the stereomicroscope (Zeiss Stemi 305 trino, New York, USA) to detect the presence of anatomical alterations, and further processed for histological examination as previously described (Power et al., 2024). Briefly, samples were cut sagittally and the two halves were placed in embedding cassettes and automatically processed (Thermo Fisher Scientific Shandon Citadel 1000, Massachusetts, USA). Three µm sections were subsequently stained with Haematoxylin and Eosin (HE) to identify possible microscopic lesions and with Grocott methenamine silver staining following the manufacturer's protocol (Bio-Optica, Milan, Italy) to detect the presence of fungi.

3. Results

3.1. Characterization of fungal isolates from *V. orientalis* nest

A total of 16 fungal isolates were obtained from different developmental stages and nest components of *V. orientalis*, including larvae, pupae, adults, and surface washes (Table 1). The isolates were identified by molecular analysis of the ITS region. Among them, the most frequently isolated taxa were yeasts and filamentous fungi commonly associated with environmental or insect-associated niches.

In the Portici (Naples) nest, six species were isolated from larvae, including *Cladosporium puyae*, *Penicillium citreosulfuratum*, *Botryotinia ranunculi*, *Aspergillus chevalieri*, *Candida parapsilosis*, and notably *Beauveria varroae* (Table 1). The latter was selected for pathogenicity testing due to its entomopathogenic potential. *Candida parapsilosis* was also recovered from pupae and adult washes, suggesting it is a common nest yeast. Yeasts such as *Metschnikowia chrysoperlae* and *M. pulcherrima* were isolated from washing water of pupae and adults, respectively, indicating contamination of external surfaces. From adult specimens, filamentous fungi such as *Penicillium citrinum*, *P. thomii*, and *Cladosporium ramotenellum* were identified (Table 1). In the Palermo nest, three fungal colonies were isolated from adults, including *Talaromyces primulinus*, *Didymella keratinophila* and *Curvularia subpappendorfii*, two fungal colonies were isolated from pupae, including *Aspergillus versicolor* and *Didymosphaeria variabile*, while no fungal colonies were isolated from larvae.

Phylogenetic analysis of the complete concatenated ITS–RPB1 dataset strongly supported the assignment of isolate PDA11Vo712 to *B. varroae* (Fig. 1). PDA11Vo712 clustered with *B. varroae* LRMH_C291 with 89% bootstrap support, and this group formed a strongly supported clade with the *B. varroae* type strain ARSEF 8257 (93% bootstrap

Table 1
Fungal species isolated from *V. orientalis*.

Source	Location	Isolate	Accession	Species*	Best match*	Query cover (%)*	Identity (%)*
Larva	Portici	PDA35Vo724	PX915584	<i>Cladosporium puyae</i>	NR_152298.1	97	99.81
Larva	Portici	PDA25Vo717	PX915585	<i>Penicillium citreosulfuratum</i>	NR_153252.1	100	99.14
Larva	Portici	PDA11Vo712	PX915586	<i>Beauveria varroae</i>	NR_111599.1	98	99.46
Larva	Portici	PDA23Vo716	PX915587	<i>Botryotinia ranunculi</i>	NR_164278.1	99	99.44
Larva	Portici	PDA31Vo719	PX915588	<i>Aspergillus chevalieri</i>	NR_135340.1	95	100
Meconium/debris	Portici	PDA13Vo713	PX915589	<i>Trichoderma atrobrunneum</i>	NR_137298.1	99	99.67
Pupa	Palermo	SAAF_2NA	PZ049847	<i>Aspergillus versicolor</i>	NR_131277.1	100	98.74
Pupa	Palermo	SAAF_2OB	PZ049849	<i>Didymosphaeria variabile</i>	NR_137006.1	93	99.65
Pupa	Portici	PDA15Vo714	PX915590	<i>Candida parapsilosis</i>	NR_130673.1	98	99.61
Pupa - Washing water	Portici	LB10Vo711	PX915591	<i>Metschnikowia chrysoperlae</i>	NR_111376.1	96	98.96
Adult	Palermo	SAAF_2CA	PZ049846	<i>Talaromyces primulinus</i>	NR_145151.1	100	98.94
Adult	Palermo	SAAF_2CC	PZ049848	<i>Didymella keratinophila</i>	NR_158275.1	100	99.94
Adult	Palermo	SAAF_2CD	PZ049850	<i>Curvularia subpapendorfii</i>	NR_147488.1	100	98.57
Adult	Portici	LB1Vo710	PX915592	<i>Penicillium citrinum</i>	NR_121224.1	93	99.4
Adult	Portici	PDA27Vo718	PX915593	<i>Penicillium thomii</i>	NR_077159.1	99	99.31
Adult-Washing water	Portici	PDA21Vo715	PX915594	<i>Metschnikowia pulcherrima</i>	NR_164379.1	100	96.25

* Species were identified on the basis of ITS sequence similarity. Searches were performed via blastn using ITS as query against ITS TYPE material db (NCBI).

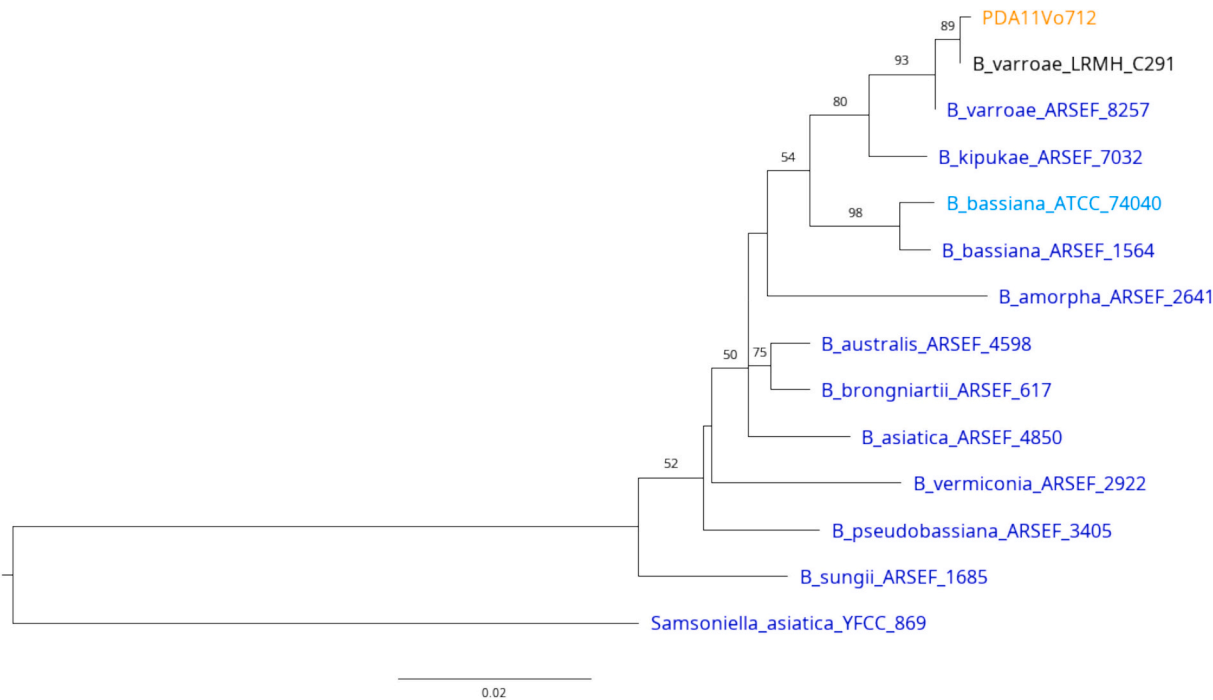


Fig. 1. Maximum-likelihood phylogenetic placement of *Beauveria varroae* isolate PDA11Vo712 based on concatenated ITS and RPB1 sequences. The phylogeny was inferred from the complete two-locus dataset comprising 14 fungal strains for which both ITS and RPB1 sequences were available. The two loci were analyzed as separate partitions using their respective best-fitting nucleotide substitution models selected according to the Bayesian Information Criterion (BIC): TIM2 + I + G4 for ITS and TrNef+G4 for RPB1. The tree was inferred using RAXML-NG. Light blue and orange labels indicate *B. bassiana* ATCC 74040 and *B. varroae* PDA11Vo712, respectively, while blue labels indicate TYPE material (reference strains). Branch support was assessed using 1000 bootstrap replicates, and bootstrap values $\geq 50\%$ are shown at the nodes. The scale bar indicates the number of nucleotide substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

support). In contrast, the *B. bassiana* strains ATCC 74040, the active ingredient of the biopesticide Naturalis®, and ARSEF 1564 formed a distinct lineage with 98% bootstrap support. The placement of PDA11Vo712 within the *B. varroae* lineage was also recovered in the broader 24-taxon concatenated analysis, which retained taxa lacking RPB1 data as missing characters, as well as in the individual ITS and RPB1 analyses (Figs. S1–S3).

3.2. Survival analysis following inoculation of conidial suspensions

Log rank analysis revealed a significant impact of treatments on *V. orientalis* survival, which was reduced in all treated groups ($n = 40$)

compared to controls ($p < 0.05$). In contact experiment (Fig. 2a), both *B. varroae* PDA11Vo712 (*Bv*) and *B. bassiana* ATCC 74040 (*Bb*) reduced survival significantly, at a similar level (Control-*Bv* log rank test: $\chi^2 = 12.24$; $P = 0.0005$; Control-*Bb* log rank test: $\chi^2 = 13.26$; $P = 0.0003$). When delivered by ingestion, *Bv* was more virulent than *Bb* (*Bb*-*Bv* log rank test: $\chi^2 = 4.80$; $P = 0.03$), with faster onset of mortality (Fig. 2b). In the indirect transmission experiment, a significant increase of mortality was observed in cage mates (Control-*Bv* log rank test: $\chi^2 = 14.31$; $P = 0.0002$; Control-*Bb* log rank test: $\chi^2 = 6.80$; $P = 0.009$), suggesting horizontal transfer by contact (Fig. 2c). Notably, mortality increased lately in indirect transmission experiments (4–5 days after treatment) compared to topical treatments (Fig. 2a).

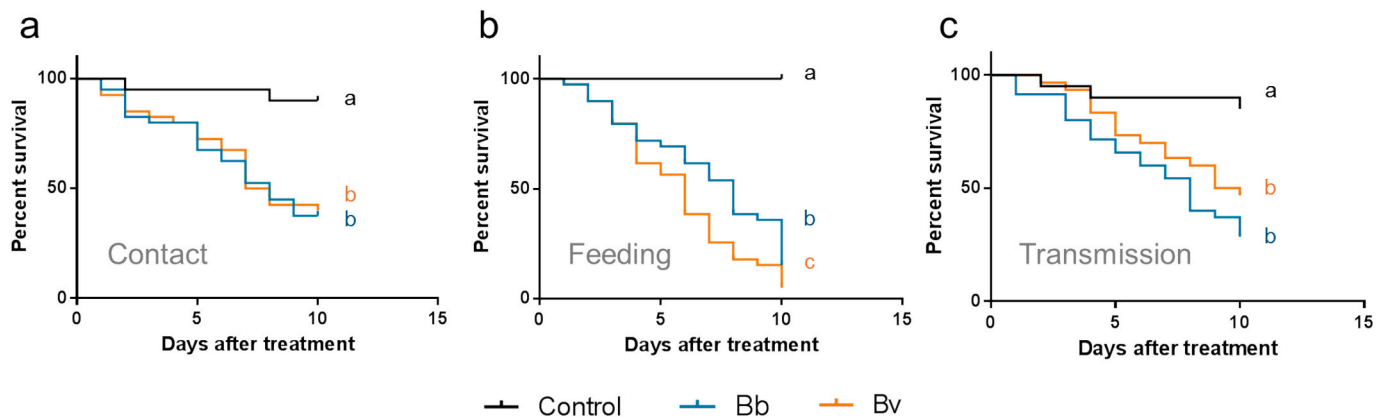


Fig. 2. Kaplan–Meier survival curves of adult hornets treated with *B. bassiana* ATCC 74040 (Bb) and *B. varroae* PDA11Vo712 (Bv) via three different inoculation methods: a) contact application; b) ingestion through sugar syrup; c) indirect transmission. Control groups received no fungal treatment. Survival was monitored daily. In each inoculation assay, subjects at risk were: Control, $n = 20$; Bb, $n = 40$; Bv, $n = 40$. Statistical differences among groups were assessed using the Log Rank (Mantel–Cox) test. Different letters indicate significant differences ($p < 0.05$).

3.3. Macroscopic and histological observations

Histological analyses were performed exclusively on hornets that survived until the end of the experimental period. Consequently, the observations reported below describe tissue alterations in surviving individuals and should not be considered representative of the full spectrum of pathological changes associated with lethal *Beauveria* infection. Macroscopic examination of hornets which survived the treatments did not show any signs of alterations in any of the observed samples. In contrast, histological examination revealed significant changes in the abdominal tissues of *Beauveria*-treated samples compared to control ones. Individuals treated with Bb (Fig. 3) and Bv (Fig. 4) showed degeneration of fat body, midgut and Malpighian tubules. Both Bb and Bv, when delivered by contact or by ingestion, successfully infected the host and showed a massive presence of hyphae (Fig. 3a and b; Fig. 4a and b), which were absent in individuals infected by indirect transmission (Fig. 3c and 4c), suggesting delayed infection. In samples

treated by indirect transmission, the recipient individuals lacked visible fungal growth; only few samples presented spores and no tissue damage was observed (Fig. 3c and 4c). Notably, some individuals challenged with Bb in the transmission test presented pyknotic nuclei in some oenocytes (Fig. 3c). The marked vehicle hornet (topically treated with either Bb and Bv) presented fungal infection in the abdomen often associated with hemocyte recruitment in the haemocoel and vacuolization of the Malpighian tubules (Fig. 5c). No tissue damage or fungal growth was observed in control samples (Fig. 3d, 4d, 5d).

Hyphal fragments and septated hyphae were even more evident in the Grocott stained samples (Fig. 5a and b), appearing as black/dark brown filaments and round/oval shaped cells. Notably, Grocott staining of samples infected by transmission showed yeast-like fungal cells (blastospores) surrounded by a light brown diffuse melanin deposit, indicating immune response of the host towards infection (Fig. 5c).

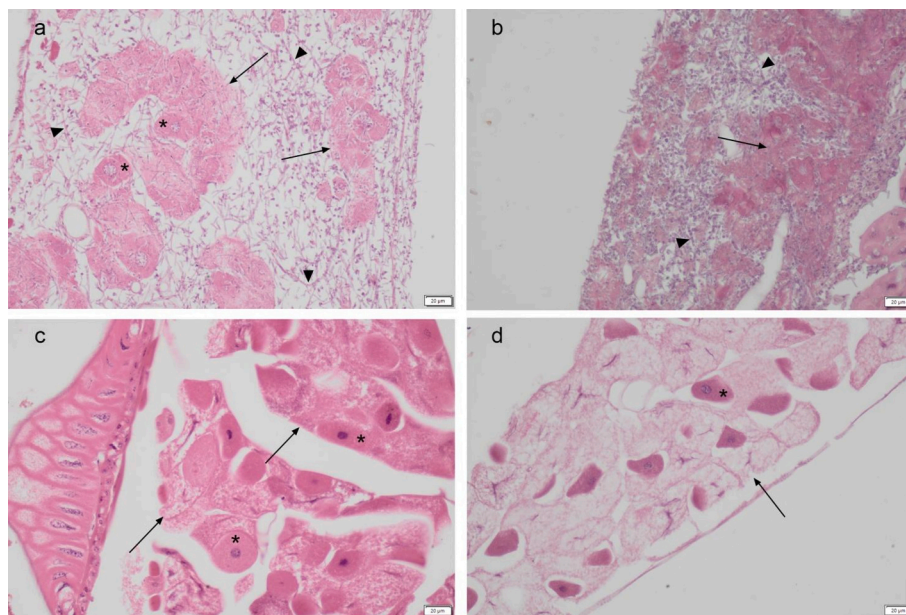


Fig. 3. *Vespa orientalis* infected by *Beauveria bassiana*. a) Individuals infected by topical administration showing fungal cells in the hemocoel (arrow heads), degenerated fat body (arrows) and oenocytes (asterisks); b) Individuals infected by oral administration showing fungi in the haemocoel (arrow heads) and degenerated fat body (arrows); c) Individuals infected by indirect transmission showing healthy trophocytes and no fungi. Nuclei of oenocytes appear pyknotic (asterisks) d) Control sample showing well developed fat body (arrow) and oenocytes (asterisk). H&E. 40 $\times$ magnification. Scale bar: 20 μ m.

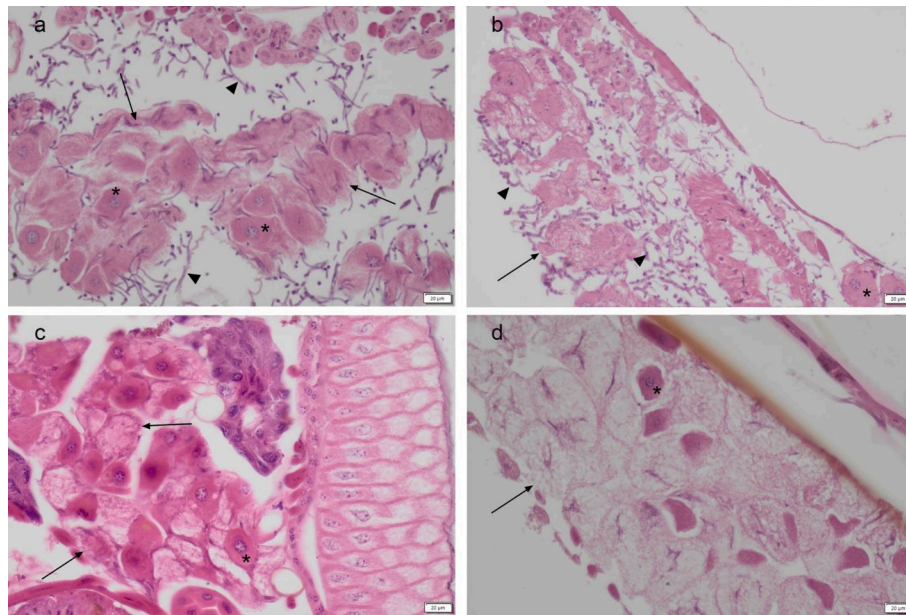


Fig. 4. *Vespa orientalis* infected by *Beauveria varroae*. a) Individuals infected by topical administration showing fungal cells in the hemocoel (arrow heads), degenerated trophocytes (arrows) and oenocytes; b) Individuals infected by oral administration showing fungi in the haemocoel (arrow heads) and degenerated fat body (arrows); c) Individuals infected by transmission showing healthy fat body (arrows) and no fungal growth d) Control sample showing well developed fat body (arrows). H&E. 40× magnification. Scale bar: 20 µm.

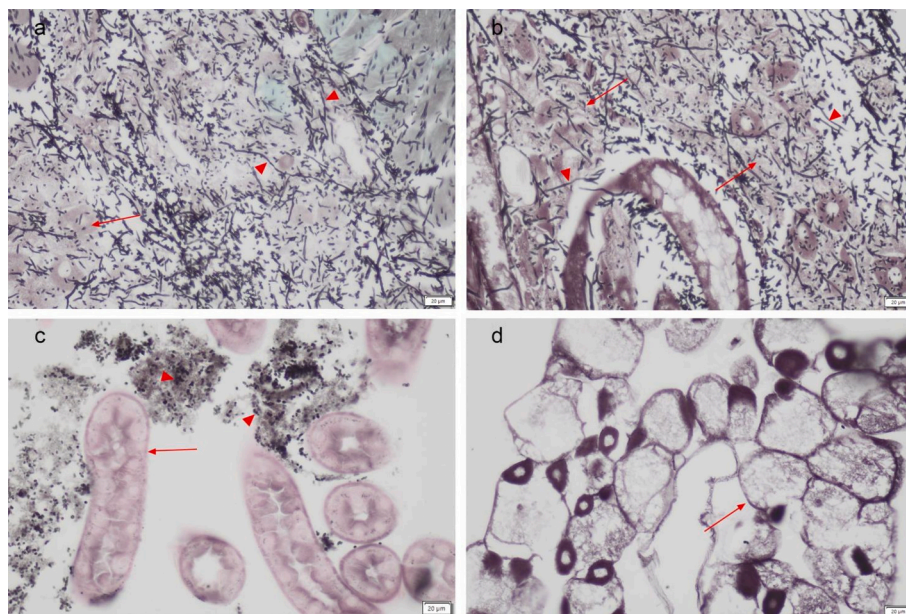


Fig. 5. Grocott staining of *Vespa orientalis* abdominal tissues. a) Individuals infected with *B. bassiana* showing hyphae in the hemocoel (arrow head) and degenerated fat bodies (arrows); b) Individuals infected with *B. varroae* showing hyphae in the haemocoel (arrow head) and degenerated fat bodies (arrows); c) Individual infected by indirect transmission showing yeast-like blastospores in the haemocoel, surrounded by a melanin deposit (arrow heads) and degeneration of Malpighian tubules; d) Control sample showing no fungal cells in hemocoel and healthy fat body (arrow). 40× magnification. Scale bar: 20 µm.

4. Discussion

In this study, we characterized the fungal community associated with *V. orientalis* decaying nests and identified *B. varroae* as a naturally occurring entomopathogen with marked virulence against adult hornets. The characterization of fungal isolates from *V. orientalis* nests revealed a diverse mycobiota comprising both filamentous fungi and yeasts commonly associated with insect habitats, nesting materials, and environmental substrates. The recovery of taxa such as *Penicillium*, *Cladosporium*, *Aspergillus*, *Botryotinia*, *Didymosphaeria*, *Talaromyces* and

Curvularia is consistent with their ubiquitous saprotrophic nature and frequent association with soil, plant material, and detritus incorporated into social insect nests (Ahmadpour et al., 2025; Batra et al., 1973; Becchimanzi and Nicoletti, 2022; Fouillaud and Morel, 1995; Guevara-Suarez et al., 2020; Nicoletti et al., 2024; Senanayake et al., 2023).

Also *Didymella keratinophila* reflects incidental acquisition from nest material or surrounding detritus. This species is of particular interest due to its ability to produce keratinase and collagenase enzymes and degrade keratin-rich substrates such as feathers in fermentation studies, indicating a saprotrophic ecology in soil and organic debris rather than

an obligate association with specific insect species or plant hosts (Al-Bedak et al., 2023; Chen et al., 2017).

The repeated isolation of yeasts including *Candida parapsilosis* and *Metschnikowia* spp. from larvae, pupae, and surface washes highlight the strong association between yeast species and insects (Stefanini, 2018; Suh et al., 2008), particularly flower visitors, including Vespidae (Jimenez et al., 2017; Stefanini et al., 2012). Similar patterns of diverse fungal and yeast communities have been described in other social Hymenoptera, where nest-associated microbiota can influence colony health either positively or negatively (Becchimanzi and Nicoletti, 2022; Mayorga-Ch et al., 2021; Turillazzi et al., 2023).

This is the first report of *B. varroae* from Vespidae, although other entomopathogenic species have been reported from wasps and hornets. In another hornet species, *Vespa velutina*, a *B. bassiana* strain was isolated from a naturally parasitized foundress (Poidatz et al., 2019). In *Polistes hebraeus* (Vespidae), the nest act as a reservoir of fungal species, including entomopathogens such as *Paecilomyces farinosus*, which may spread in the environment after the seasonal colony collapse (Fouillaud and Morel, 1995). Our findings align with previous studies (Rose et al., 1999), supporting the hypothesis that hornet nests are not just reservoirs of environmental microbes but also arenas for natural host–pathogen interactions.

These results represent the first characterization of fungal communities associated with *V. orientalis* nests, adults, and larvae, which demonstrates that several fungal organisms are able to colonize the oriental hornet's microcosm. This provides insights into the microbial ecology of *V. orientalis*, useful for understanding synergistic or antagonistic interactions and, therefore, for developing biopesticide-based pest management strategies.

In contrast to *B. bassiana*, globally used as biopesticide (Mascarin and Jaronski, 2016), *B. varroae* has only recently been identified as a potential biocontrol agent, with very limited studies conducted so far (Lagogiannis et al., 2023; Mantzoukas et al., 2025, 2023). In one study, among 15 isolates tested, a *B. varroae* native strain showed the highest toxicity to all *Halyomorpha halys* (Hemiptera: Pentatomidae) stages (Mantzoukas et al., 2023). When delivered by spraying, *B. varroae* strains resulted effective also against larval stages of Notodontidae and Noctuidae (Lepidoptera) (Lagogiannis et al., 2023; Mantzoukas et al., 2025).

The concordance among single-locus and concatenated phylogenies supports the assignment of PDA11Vo712 to *B. varroae* and its distinction from the closely related *B. kipukae* and *B. bassiana*. While ITS alone provided limited resolution among these closely related lineages, the additional phylogenetic signal from RPB1 and the partitioned ITS–RPB1 analysis considerably strengthened the taxonomic placement of our isolate. These results highlight the value of combining independent molecular markers for resolving closely related and potentially cryptic *Beauveria* species (Bustamante et al., 2019; Becchimanzi et al., 2021). The genetic distinction between PDA11Vo712 and the commercial ATCC 74040 strain (Naturalis®) is partially reflected in biological performance.

Both fungi significantly reduced *V. orientalis* survival when delivered by contact, demonstrating that hornet adults are susceptible to infection by *Beauveria* spp. under laboratory conditions. Although the use of field-collected hornets inevitably introduced some degree of biological variability, all individuals were sampled simultaneously while actively foraging at the same apiaries, thereby likely reducing age-related variation. Moreover, the statistically significant differences in survival observed indicate that the pathogenic effects of *Beauveria* were sufficiently robust to be detected despite the unavoidable heterogeneity of wild-caught individuals. Our findings are in line to what reported by Poztdaz et al. (2018), who inoculated adult hornets with one *B. bassiana* and four *Metarhizium robertsii* strains using different routes of delivery. Contact treatment using the same concentration that we used in the present study (10^7 spores per milliliter), led to reduced survival by half after only five to six days, in agreement with our results. Moreover, no

significant difference in mortality was reported when comparing different strains administered by contact (Poidatz et al., 2018), similarly to what we observed. Nevertheless, differences emerged when alternative delivery routes were tested.

Although *B. bassiana* is considered a contact pathogen, several studies indicate that oral exposure can induce significant insect mortality, even without evidence of gut infection (Mannino et al., 2019; Veen, 1966; Yanagita and Iwashita, 1987). Orally ingested conidia generally do not germinate in the midgut, which is a hostile environment, but may adhere to and germinate on the oral cavity or foregut structures (Jeffs et al., 1997; Lai et al., 2024). This mechanism, together with possible toxic or immunomodulatory effects of fungal metabolites (Mannino et al., 2019), likely explains the high mortality observed in hornets following oral delivery.

Indeed, in our study, ingestion of both *B. bassiana* and *B. varroae* increased mortality, accompanied by tissue invasion, suggesting that oral exposure can, under certain conditions, lead to successful systemic infection. However, toxin-mediated effects cannot be excluded. *Beauveria* species synthesize a wide array of secondary metabolites, including cyclic peptides (beauvericin, bassianolide), polyketides, and quinones, that are linked to virulence and host immune modulation (Feng et al., 2015; Pedrini, 2022). These compounds have been identified in culture extracts and infected insects and may be present in conidial preparations used for inoculation (Pedrini, 2022), potentially contributing to host mortality following oral exposure. Future studies will elucidate the mechanism of the observed oral activity by performing metabolomic profiling and bioassays including heat-killed conidia and filtered culture supernatant. When delivered by ingestion, *Bv* PDA11Vo712 resulted in higher mortality than *Bb* ATCC 74040. A similar result was also observed in the study by Poidatz et al. on *V. velutina*, reporting that entomopathogenic strains can differ in their oral activity (Poidatz et al., 2018). Our results suggest that *B. varroae*, isolated directly from the hornet larvae, may be better adapted to exploit physiological conditions encountered following oral exposure, such as gut environment, immune barriers, or hemolymph composition.

A limitation of the oral infection assay is that the actual amount of conidia ingested by individual hornets could not be quantified because food consumption was not measured. Although all individuals were provided with the same freshly prepared sucrose solution containing a standardized concentration of viable conidia under identical experimental conditions, variation in feeding behavior may have resulted in differences in the effective inoculum acquired by each hornet. Consequently, the oral assay should be interpreted as evaluating the pathogenic potential of *Beauveria* following oral exposure rather than defining a precise dose-response relationship. Transmission experiments further showed that both fungi can induce mortality in untreated cage mates, indicating potential for horizontal transfer through contact, as reported for *V. velutina* (Poidatz et al., 2018). The delayed mortality and lack of overt tissue colonization in recipient hornets are consistent with indirect fungal exposure through contact with contaminated individuals, although the amount of conidia transferred was not quantified in the present study. Importantly, histological analyses were performed on individuals that survived until the end of the experimental period, allowing the evaluation of tissue alterations occurring from fungal infection rather than post-mortem autolytic mechanisms. Moreover, the absence of extensive fungal colonization in these survivors suggests that infection remained localized or was effectively controlled, although we cannot exclude that some individuals experienced lower levels of exposure. This is in line with the evolutionary history of *Beauveria* species, which are considered generalist opportunistic pathogens whose interactions with the host are comparable to predators that cull the weakest prey (Boomsma et al., 2014). The presence of melanized deposits enveloping yeast-like fungal cells in surviving individuals further supports immune recognition and control rather than active systemic mycosis. Our histological analyses provided important unprecedented insights into the interaction between *Beauveria* spp. and the hornet

immune system. Following contact or ingestion, both *B. bassiana* and *B. varroae* successfully invaded host tissues, with extensive degeneration of fat body, midgut, and Malpighian tubules. The massive presence of hyphal bodies and septate hyphae in these tissues indicates active proliferation within the hemocoel and is consistent with the canonical infection cycle of entomopathogenic fungi. Particularly, degeneration of the fat bodies observed in both administration modalities suggests a possible targeted action of fungal enzymes towards the fat body. The fat body in insects plays a role similar to the liver and the adipose tissue in mammals: it is involved in nutrient storage, it regulates hormonal and nutritional signals (Arrese and Soulagues, 2010), and participates in immune response by secreting several effectors, including antimicrobial peptides (Hoffmann, 1995; Wrońska et al., 2023). Therefore, targeting the fat body could on one hand release nutrients required for the development of the fungi, while on the other it could impair the immune response able to fight the infection, representing an efficient strategy for a successful parasitization. In contrast, transmission treatments revealed a markedly different pattern. Recipient hornets generally lacked visible tissue invasion, and Grocott staining highlighted the presence of yeast-like fungal cells (blastospores) surrounded by diffuse melanin deposits. This melanization response suggests that the hornet immune system recognized the pathogen activating a response. The formation of melanized clots in the hemocoel is a hallmark of insect innate immunity (Hoffmann, 1995) and likely contributed to the delayed and reduced pathogenic effects observed in transmission assays. Possibly, a lower-infective dose resulting from a “dilution-effect” from the carrier individual to the recipient ones could trigger immune response and effectively fight the fungi. Nevertheless, because histological analyses were restricted to individuals that survived until the end of the experimental period, our observations likely underestimate the extent of tissue damage associated with progressive or lethal infections. Consequently, the absence of widespread fungal colonization in these specimens should not be interpreted as evidence that systemic infection did not occur in individuals that succumbed earlier during the experiment.

Also, to date it is not clear if hornets hold immune priming, which could reduce susceptibility to the fungus, delay infection and increase survival rate of individuals primed with a low amount of inoculum (Cooper and Eleftherianos, 2017). Immune priming involves an enhanced immune response following a “first contact” with a sublethal dosage of a pathogen (or pathogen derivatives), resulting in a higher resistance to a “second encounter” with the pathogen (Lanz-Mendoza and Contreras-Garduño, 2022). Immune priming can be within-generation, trans-stadial and trans-generational immune priming, particularly developed in social insects showing high parental care and can be life-stage related (Lanz-Mendoza and Contreras-Garduño, 2022). Previous studies using entomopathogens have shown contrasting data underlining the existence of species-specific responses. Particularly, when challenging *Formica selys* with *B. bassiana* no evidence for immune priming was observed (Reber and Chapuisat, 2012); on the contrary Sadd and Schmid-Hempel (Sadd and Schmid-Hempel, 2006) proved that *Bombus terrestris* presented increased survival rates when challenged with homologous secondary infections by *Paenibacillus alvei* and *P. larvae*. Although our experimental design did not allow us to directly assess immune priming, the melanized deposits surrounding yeast-like fungal cells observed in recipient hornets indicate activation of cellular immune defenses and fungal encapsulation. Nevertheless, because neither circulating hemocyte abundance nor antimicrobial peptide expression was quantified, the contribution of immune priming to the reduced susceptibility observed after indirect exposure remains speculative and warrants further investigation. Immune priming and more in general immune response in Vespidae remain poorly characterized, with only a few recent studies providing initial insights (Cappa et al., 2022; Meriggi et al., 2019), representing interesting topics for future studies.

Our observations suggest that infection outcome in *V. orientalis* depends strongly on the route of exposure. While direct inoculation

overwhelms host defenses, low-dose exposure acquired through social contact may trigger immune containment rather than lethal infection. This distinction is critical when extrapolating laboratory results to more complex ecological contexts. Consequently, the development of effective biocontrol strategies requires a detailed understanding of dose–response relationships under different exposure scenarios, as well as of the mechanisms governing conidial transfer from donor to recipient hornets. In this regard, inoculum acquisition and transmission may be enhanced by incorporating conidia into sugar- or protein-based baits to increase attractiveness (de la Hera et al., 2024; El-Gohary et al., 2026; Rojas-Nossa et al., 2024). Although nestmates were not avoided after topical treatment with fungal biopesticides in other vespid species (De Souza et al., 2023), information on the behavioral effects of biopesticides in *V. orientalis* is still scarce. Within this framework, trophallaxis (Hunt et al., 1982) and grooming (Pusceddu et al., 2022) represent key traits that warrant further investigation for the development of novel and effective delivery strategies.

A limitation of this study is that entomopathogenic strains were evaluated exclusively under laboratory conditions and only on adult hornets. Future research should therefore focus on identifying the mechanisms and bottlenecks governing conidial transfer from foraging adults to the nest, with the aim of developing delivery systems that exploit social interactions to disseminate the pathogen within the colony. Future studies should, for example, quantify the efficiency of conidial transfer under different social contexts, including varying donor-to-recipient ratios, group sizes, and contact frequencies, to identify the conditions that maximize pathogen dissemination within colonies. Likewise, behavioral investigations examining interactions between contaminated and uncontaminated nestmates, including grooming, trophallaxis, avoidance, and other social behaviours, would clarify how these processes influence fungal transmission and ultimately determine the success of “Trojan-horse” delivery strategies. Such strategies at the nest level would require formulations that maximize hornet acquisition and ingestion while minimizing access by non-target organisms, for example through species-specific sugar- or protein-based baits (Lester, 2025; Rojas-Nossa et al., 2018; Turchi and Derijard, 2018). Moreover, the effectiveness and environmental safety of these approaches will depend on optimizing bait station design and deployment timing to coincide with periods of intense hornet foraging activity while limiting exposure of non-target insects. Comprehensive field evaluations will therefore be essential before operational implementation of this strategy. Recent advances in the rearing of hornets under controlled conditions provide new opportunities to assess the efficacy of entomopathogenic fungi in more realistic experimental frameworks that incorporate social behavior, nest dynamics, and multiple life stages (Van Itterbeeck et al., 2021).

Taken together, our results support the potential of *Beauveria* spp. as candidate biocontrol agents against *V. orientalis*, particularly in light of their high virulence across different inoculation routes. Notably, *B. varroae*, isolated directly from *V. orientalis* larvae, represents a promising alternative to commercial *B. bassiana* strains, as it may be better adapted to the hornet host and nest environment. Histological analyses revealed extensive tissue invasion and host immune responses, highlighting route-dependent infection dynamics. Transmission experiments further indicated that indirect exposure can induce mortality while eliciting immune containment in surviving individuals. Together, these findings support the feasibility of exploiting entomopathogenic fungi for the control of *V. orientalis*. Importantly, the isolation of a nest-associated *Beauveria* species suggests opportunities for more specific and ecologically informed biocontrol strategies. Overall, our results provide a foundation for the development of targeted, sustainable fungal-based approaches to mitigate the impact of this emerging beekeeping pest.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve the clarity, grammar, and readability of selected sections of the manuscript. The authors carefully reviewed and edited all AI-generated output, verified its accuracy, and take full responsibility for the content of the published article.

CRedit authorship contribution statement

Ernesto Ragusa: Writing – original draft, Investigation, Conceptualization. **Karen Power:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Livio Torta:** Writing – review & editing, Writing – original draft, Investigation. **Marika Lamendola:** Investigation. **Giovanni Jesu:** Investigation. **Giovanna De Leva:** Investigation. **Alessandro Scavuzzo:** Investigation. **Laura Bortolotti:** Supervision, Funding acquisition, Formal analysis. **Andrea Becchimanzi:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval

Not applicable.

Funding

This work was supported by GENAPIS.IT.4 Project, funded by the Italian Ministry of Agriculture under REG. UE 2021/2115 (CAMPAGNA 2026N63730000138).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Claire Power for helping editing figures. The authors acknowledge the Histopathology and Diagnostics Core at Department of Biology of University of Naples “Federico II” for their technical and scientific support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jip.2026.108728>.

Data availability

Sequence data supporting the findings of this study are available in the GenBank database through the accession numbers provided in Table 1 and Table S1. The phylogenetic tree files (.nexus) and the survival dataset are available in the Zenodo repository, <https://doi.org/10.5281/zenodo.22026619>.

References

- Abd Al-Fattah, M.A.A.-W., Nour, M.E., Aziz, D.A., Marzouk, W.M., 2014. New applications for protecting honeybee, *Apis mellifera* L., colonies from attacking the oriental hornet (*Vespa orientalis* fab.). Egypt. Acad. J. Biol. Sci. A, Entomol. <https://doi.org/10.21608/eajbsa.2014.13201>.
- Ahmadpour, A., Heidarian, Z., Ghosta, Y., Alavi, Z., Alavi, F., Manamgoda, D.S., Karunarathna, S.C., Rampelotto, P.H., 2025. Morphological and molecular characterization of *Curvularia* species from Iran, with description of two novel species and two new records. Mycologia 117, 261–285. <https://doi.org/10.1080/00275514.2025.2450754>.
- Al-Bedak, O.A.-H.M., Moharram, A.M., Hussein, N.A.-G., Taha, D.M., Stephenson, S.L., Ameen, F., 2023. Microbial exploitation of feather wastes for sustainable production of keratinase and collagenase enzymes by *Didymella keratinophila* AUMC 15399 in submerged fermentation. Fermentation 9, 507. <https://doi.org/10.3390/fermentation9060507>.
- Arca, M., Mougél, F., Guillemaud, T., Dupas, S., Rome, Q., Perrard, A., Muller, F., Fossoud, A., Capdevielle-Dulac, C., Torres-Leguizamon, M., Chen, X.X., Tan, J.L., Jung, C., Villemant, C., Arnold, G., Silvain, J.-F., 2015. Reconstructing the invasion and the demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe. Biol. Invasions 17, 2357–2371. <https://doi.org/10.1007/s10530-015-0880-9>.
- Archer, M., 1998. Taxonomy, distribution and nesting biology of *Vespa orientalis* L. (HYM., VESPIDAE). Entomol. Mon. Mag. 134, 45–51.
- Arrese, E.L., Soulages, J.L., 2010. Insect fat body: energy, metabolism, and regulation. Annu. Rev. Entomol. 55, 207–225. <https://doi.org/10.1146/annurev-ento-112408-085356>.
- Baracchi, D., Cusseau, G., Pradella, D., Turillazzi, S., 2010. Defence reactions of *Apis mellifera ligustica* against attacks from the European hornet *Vespa crabro*. Ethol. Ecol. Evol. 22, 281–294. <https://doi.org/10.1080/03949370.2010.502323>.
- Batra, L.R., Batra, S.W.T., Bohart, G.E., 1973. The mycoflora of domesticated and wild bees (Apoidea). Mycopathol. Mycol. Appl. 49, 13–44. <https://doi.org/10.1007/BF02057445>.
- Bava, R., Castagna, F., Piras, C., Musolino, V., Lupia, C., Palma, E., Britti, D., Musella, V., 2022. Entomopathogenic Fungi for pests and predators control in beekeeping. Vet. Sci. 9, 95. <https://doi.org/10.3390/vetsci9020095>.
- Becchimanzi, A., Nicoletti, R., 2022. Aspergillus-bees: a dynamic symbiotic association. Front. Microbiol. 13.
- Becchimanzi, A., Zimowska, B., Nicoletti, R., 2021. Cryptic diversity in *Cladosporium cladosporioides* resulting from sequence-based species delimitation analyses. Pathogens 10, 1167. <https://doi.org/10.3390/pathogens10091167>.
- Beggs, J.R., Brockerhoff, E.G., Corley, J.C., Kenis, M., Masciocchi, M., Muller, F., Rome, Q., Villemant, C., 2011. Ecological effects and management of invasive alien Vespidae. BioControl 56, 505–526. <https://doi.org/10.1007/s10526-011-9389-z>.
- Boomsma, J.J., Jensen, A.B., Meyling, N.V., Eilenberg, J., 2014. Evolutionary interaction networks of insect pathogenic fungi. Annu. Rev. Entomol. 59, 467–485. <https://doi.org/10.1146/annurev-ento-011613-162054>.
- Bressi, N., Colla, A., Tomasin, G., 2019. Orientali verso nord: insediamento di una popolazione urbana di Calabrone Orientale (*Vespa orientalis* Linnaeus, 1771) a Trieste, NE Italy (Hymenoptera, Vespidae). Atti Mus. Civ. Storia Nat. Trieste 60, 273–275.
- Brodeur, J., 2012. Host specificity in biological control: insights from opportunistic pathogens. Evol. Appl. 5, 470–480. <https://doi.org/10.1111/j.1752-4571.2012.00273.x>.
- Bustamante, D.E., Oliva, M., Leiva, S., Mendoza, J.E., Bobadilla, L., Angulo, G., Calderon, M.S., 2019. Phylogeny and species delimitations in the entomopathogenic genus *Beauveria* (Hypocreales, Ascomycota), including the description of *B. Peruviansis* sp. nov. MycoKeys 58, 47–68. <https://doi.org/10.3897/mycokeys.58.35764>.
- Cappa, F., Cini, A., Meriggi, N., Poidatz, J., Thiéry, D., Cervo, R., 2022. Immune competence of the invasive hornet *Vespa velutina* and its native counterpart *Vespa crabro*: a comparison across caste and sex. Entomol. Gen. 42, 11–20. <https://doi.org/10.1127/entomologia/2021/1301>.
- Ceccolini, F., 2022. Review of the occurrence of the oriental hornet *Vespa orientalis* Linnaeus, 1771 in the islands of Greece (Hymenoptera: Vespidae: Vespinae). Entomol. Hell. 31, 41–49. <https://doi.org/10.12681/eh.28762>.
- Cetkovic, A., 2003. Review of the European distribution of the oriental hornet (Hymenoptera, Vespidae: *Vespa orientalis* L.). Ekologija 37, 1–22.
- Chen, Q., Hou, L.W., Duan, W.J., Crous, P.W., Cai, L., 2017. *Didymellaceae* revisited. Stud. Mycol. 87, 105–159. <https://doi.org/10.1016/j.simyco.2017.06.002>.
- Cooper, D., Eleftherianos, I., 2017. Memory and specificity in the insect immune system: current perspectives and future challenges. Front. Immunol. 8, 539.
- Cubero, O.F., Crespo, A., Fatehi, J., Bridge, P.D., 1999. DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. Plant Syst. Evol. 216, 243–249. <https://doi.org/10.1007/BF01084401>.
- Darchen, R., 1964. Biologie de *Vespa orientalis*. Les premiers stades de développement. Insect. Soc. 11, 141–157. <https://doi.org/10.1007/BF02222934>.
- Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., Flouri, T., 2020. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. Mol. Biol. Evol. 37, 291–294. <https://doi.org/10.1093/molbev/msz189>.
- De Souza, A.R., Prato, A., Franca, W., Santos, S., Lima, L.D., Alves, D.A., Bernardes, R.C., Santos, E.F., Do Nascimento, F.S., Lima, M.A.P., 2023. A predatory social wasp does not avoid nestmates contaminated with a fungal biopesticide. Environ. Sci. Pollut. Res. 30, 103851–103861. <https://doi.org/10.1007/s11356-023-29770-5>.
- Dvořák, L., 2006. Oriental hornet *Vespa orientalis* Linnaeus, 1771 found in Mexico (Hymenoptera, Vespidae, Vespinae). Entomol. Probl. 36, 80.
- Edgar, R.C., 2022. Muscle5: high-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. Nat. Commun. 13, 6968. <https://doi.org/10.1038/s41467-022-34630-w>.
- El-Gohary, S.G., Mohsen, A.E.-A.M.A., Youssif, M.A.I., Alkeridis, L.A., Al-Shuraym, L.A., Sayed, S., Shukry, M., Helaly, S.M.M.Y., 2026. Seasonal dynamics and nest characterization of *Vespa orientalis* (Hymenoptera: Vespidae) in apiaries: insights from bait trap capture efficiency. Insects 17, 58. <https://doi.org/10.3390/insects17010058>.
- Feng, P., Shang, Y., Cen, K., Wang, C., 2015. Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc. Natl. Acad. Sci. USA 112, 11365–11370. <https://doi.org/10.1073/pnas.1503200112>.

- Fouillaud, M., Morel, G., 1995. Fungi associated with nests of the paper wasp *Polistes hebraeus* (Hymenoptera: Vespidae) on La Reunion Island. *Environ. Entomol.* 24, 298–305.
- Fuguet, R., Vey, A., 2004. Comparative analysis of the production of insecticidal and melanizing macromolecules by strains of *Beauveria* spp.: in vivo studies. *J. Invertebr. Pathol.* 85, 152–167. <https://doi.org/10.1016/j.jip.2004.03.001>.
- Gereys, B., Coache, A., Filippi, G., 2021. Présence en France métropolitaine d'un frelon allochtone : *Vespa orientalis* Linnaeus, 1771 (Le Frelon oriental) (Hymenoptera, Vespidae, Vespinae). *Faunitaxys* 9, 1–5.
- Graziani, F., Cianferoni, F., 2021. The northernmost record of *Vespa orientalis* Linnaeus, 1771 (Hymenoptera: Vespidae) in peninsular Italy. *Rev. Gaditana Entomol.* 12, 173–178.
- Guevara-Suarez, M., García, D., Cano-Lira, J.F., Guarro, J., Gené, J., 2020. Species diversity in penicillium and Talaromyces from herbivore dung, and the proposal of two new genera of penicillium-like fungi in Aspergillaceae. *Fungal Syst. Evol.* 5, 39–75. <https://doi.org/10.3114/fuse.2020.05.03>.
- Hajek, A.E., St. Leger, R.J., 1994. Interactions between fungal pathogens and insect hosts. *Annu. Rev. Entomol.* 39, 293–322. <https://doi.org/10.1146/annurev.en.39.010194.001453>.
- Hanna, C., Foote, D., Kremen, C., 2014. Competitive impacts of an invasive nectar thief on plant–pollinator mutualisms. *Ecology* 95, 1622–1632. <https://doi.org/10.1890/13-1276.1>.
- de la Hera, O., Quintanilla-Casas, B., Bro, R., Fañanas, R., Alonso, R.M., 2024. Volatile organic compound profile for the search of rejection markers in protein baits used as *Vespa velutina* control method. *Microchem. J.* 207, 111685. <https://doi.org/10.1016/j.microc.2024.111685>.
- Hernández, S., Lanz, H., Rodríguez, M.H., Torres, J.A., Adolfo, M.-P., Tsutsumi, V., 1999. Morphological and cytochemical characterization of female *Anopheles albimanus* (Diptera: Culicidae) hemocytes. *J. Med. Entomol.* 36, 426–434.
- Hoffmann, J.A., 1995. Innate immunity of insects. *Curr. Opin. Immunol.* 7, 4–10. [https://doi.org/10.1016/0952-7915\(95\)80022-0](https://doi.org/10.1016/0952-7915(95)80022-0).
- Hunt, J.H., Baker, I., Baker, H.G., 1982. Similarity of amino acids in nectar and larval saliva: the nutritional basis for trophallaxis in social wasps. *Evolution* 36, 1318–1322. <https://doi.org/10.2307/2408164>.
- Ishay, J., 1964. Observations sur la biologie de la guêpe orientale *Vespa orientalis* F. *Insect. Soc.* 11, 193–206. <https://doi.org/10.1007/BF02222673>.
- Ishay, J., Ikan, R., 1968. Food exchange between adults and larvae in *Vespa orientalis* F. *Anim. Behav.* 16, 298–303. [https://doi.org/10.1016/0003-3472\(68\)90013-4](https://doi.org/10.1016/0003-3472(68)90013-4).
- Jeffs, L.B., Feng, M.-G., Falkowsky, J.E., Khachatourians, G.G., 1997. Infection of the migratory grasshopper (Orthoptera: Acrididae) by ingestion of the entomopathogenic fungus *Beauveria bassiana*. *J. Econ. Entomol.* 90, 383–390. <https://doi.org/10.1093/jeet/90.2.383>.
- Jimenez, S.I., Carroll, C., Babcock, T., Derstine, N., Hadwin, A., Moore, M., Gries, G., 2017. Yeasts Harbored by vespine wasps in the Pacific northwest. *Environ. Entomol.* 46, 217–225. <https://doi.org/10.1093/ee/nvw173>.
- Kim, J.-K., Choi, M., Moon, T.-Y., 2006. Occurrence of *Vespa velutina* Lepeletier from Korea, and a revised key for Korean *Vespa* species (Hymenoptera: Vespidae). *Entomol. Res.* 36, 112–115. <https://doi.org/10.1111/j.1748-5967.2006.00018.x>.
- Kozlov, A.M., Darriba, D., Flouri, T., Morel, B., Stamatakis, A., 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35, 4453–4455. <https://doi.org/10.1093/bioinformatics/btz305>.
- Lagogiannis, I., Mantzoukas, S., Eliopoulos, P.A., Poulas, K., 2023. First record of *Beauveria varroae*, cordyceps blackwelliae, and Purpureocillium lavenderum from Greece and their pathogenicity against Thaumetopoea pityocampa. *Diversity* 15, 312. <https://doi.org/10.3390/d15030312>.
- Lai, Y., Zheng, W., Zheng, Y., Lu, H., Qu, S., Wang, L., Li, M., Wang, S., 2024. Unveiling a novel entry gate: insect foregut as an alternative infection route for fungal entomopathogens. *Int. J. Hydrog. Energ.* 5, 100644. <https://doi.org/10.1016/j.xinn.2024.100644>.
- Lanz-Mendoza, H., Contreras-Garduño, J., 2022. Innate immune memory in invertebrates: concept and potential mechanisms. *Dev. Comp. Immunol.* 127, 104285. <https://doi.org/10.1016/j.dci.2021.104285>.
- Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30, 3276–3278. <https://doi.org/10.1093/bioinformatics/btu531>.
- Lester, P.J., 2025. Recent advances and avenues for the pest management of invasive social wasps and hornets. *Curr. Opin. Insect Sci.* 68, 101336. <https://doi.org/10.1016/j.cois.2025.101336>.
- Malaquias Souto, P., Santos, S.S., Sarmento, A., de Liz Ronsani, A., Tomaz, A.L., Azevedo-Pereira, H.M.V.S., Leston, S., Ramos, F., Sousa, J.P., Capela, N., 2025. Assessing the oral toxicity of acetamiprid, spinosad, cypermethrin, and pyrethrins in the invasive hornet *Vespa velutina* nigrithorax. *Sci. Rep.* 15, 45112. <https://doi.org/10.1038/s41598-025-31988-x>.
- Mannino, M.C., Huarte-Bonnet, C., Davyt-Colo, B., Pedrini, N., 2019. Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic Fungi. *J. Fungi* 5, 33. <https://doi.org/10.3390/jof5020033>.
- Mantzoukas, S., Koutsogeorgiou, E.I., Lagogiannis, I., Gogolashvili, N., Fifi, G.T., Navrozidis, E.I., Thomidis, T., Andreadis, S.S., 2023. Effect of entomopathogenic Fungi to eggs and nymphs survival of Halyomorpha halys (Hemiptera: Pentatomidae) under laboratory conditions. *Curr. Microbiol.* 81, 48. <https://doi.org/10.1007/s00284-023-03547-y>.
- Mantzoukas, S., Papantzikos, V., Lagogiannis, I., Eliopoulos, P.A., Patakioutas, G., 2025. Biocontrol potential of a new *Beauveria varroae* strain isolated from an urban ecosystem against two species of Noctuidae pests. *Crops* 5, 49. <https://doi.org/10.3390/crops5040049>.
- Mascarin, G.M., Jaronski, S.T., 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. *World J. Microbiol. Biotechnol.* 32, 177. <https://doi.org/10.1007/s1274-016-2131-3>.
- Matsuura, M., Yamane, S., Sakagami, S.F., Matsuura, M., 1990. *Biology of the Vespine Wasps: With 104 Figures [Aus d. Japan. übers.]*. Springer, Berlin Heidelberg.
- Mayorga-Ch, D., Rodríguez-C, C., Ortiz-Reyes, A., Romero-Tabarez, M., Sarmiento, C.E., 2021. Interactions of social wasps with microorganisms. In: Prezoto, F., Nascimento, F.S., Barbosa, B.C., Somavilla, A. (Eds.), *Neotropical Social Wasps*. Springer International Publishing, Cham, pp. 405–434. https://doi.org/10.1007/978-3-030-53510-0_22.
- Meriggi, N., Di Paola, M., Vitali, F., Rivero, D., Cappa, F., Turillazzi, F., Gori, A., Dapporto, L., Beani, L., Turillazzi, S., Cavalieri, D., 2019. *Saccharomyces cerevisiae* induces immune enhancing and shapes gut microbiota in social wasps. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.02320>.
- Nicoletti, R., Russo, E., Becchimanzi, A., 2024. Cladosporium—insect relationships. *J. Fungi* 10, 78. <https://doi.org/10.3390/jof10010078>.
- Otis, G.W., Taylor, B.A., Mattila, H.R., 2023. Invasion potential of hornets (Hymenoptera: Vespidae: *Vespa* spp.). *Front. Insect Sci.* 3. <https://doi.org/10.3389/finsc.2023.1145158>.
- Pedrini, N., 2022. The entomopathogenic fungus *Beauveria bassiana* shows its toxic side within insects: expression of genes encoding secondary metabolites during pathogenesis. *J. Fungi* 8, 488. <https://doi.org/10.3390/jof8050488>.
- Poidatz, J., López Plantey, R., Thiéry, D., 2018. Indigenous strains of *Beauveria* and *Metharizium* as potential biological control agents against the invasive hornet *Vespa velutina*. *J. Invertebr. Pathol.* 153, 180–185. <https://doi.org/10.1016/j.jip.2018.02.021>.
- Poidatz, J., Plantey, R.J.L., Thiéry, D., 2019. A *Beauveria bassiana** strain naturally parasitizing the bee predator *Vespa velutina** in France. *Entomol. Gen.* 73–79. <https://doi.org/10.1127/entomologia/2019/0690>.
- Poidatz, J., Lihoreau, M., Thiéry, D., 2022. Patterns of food transfer in yellow-legged hornet nests revealed by heavy metal tracers. *Entomol. Gen.* 809–817. <https://doi.org/10.1127/entomologia/2022/1339>.
- Power, K., Altamura, G., Martano, M., Maiolino, P., 2022. Detection of honeybee viruses in *Vespa orientalis**. *Front. Cell. Infect. Microbiol.* 12.
- Power, K., Martano, M., Ragusa, E., Altamura, G., Maiolino, P., 2023. Detection of honey bee viruses in larvae of *Vespa orientalis*. *Front. Cell. Infect. Microbiol.* 13. <https://doi.org/10.3389/fcimb.2023.1207319>.
- Power, K., Cilia, G., Ragusa, E., Rizzo, R., Bortolotti, L., Maiolino, P., 2024. Occurrence of *Nosema ceranae*, *Ascosphaera apis* and trypanosomatids in *Vespa orientalis* Linnaeus 1771. *J. Invertebr. Pathol.* 206, 108168. <https://doi.org/10.1016/j.jip.2024.108168>.
- Pusceddu, M., Ledzzeri, M., Cocco, A., Floris, I., Satta, A., 2022. Bio-ethology of *Vespa crabro* in Sardinia (Italy), an area of new introduction. *Biology* 11, 518. <https://doi.org/10.3390/biology11040518>.
- Quesada-Moraga, E., Vey, A., 2003. Intra-specific variation in virulence and in vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of in vivo and in vitro passage on these factors. *Biocontrol Sci. Tech.* 13, 323–340. <https://doi.org/10.1080/0958315031000110346>.
- Ranesi, M., Vitale, S., Staropoli, A., Di Lelio, I., Izzo, L.G., De Luca, M.G., Becchimanzi, A., Pennacchio, F., Lorito, M., Woo, S.L., Vinale, F., Turrà, D., 2024. Field isolates of *Beauveria bassiana* exhibit biological heterogeneity in multitrophic interactions of agricultural importance. *Microbiol. Res.* 286, 127819. <https://doi.org/10.1016/j.micres.2024.127819>.
- Reber, A., Chapuisat, M., 2012. No evidence for immune priming in ants exposed to a fungal pathogen. *PLoS One* 7, e35372. <https://doi.org/10.1371/journal.pone.0005372>.
- Richards, O.W., 1971. The biology of the social wasps (hymenoptera, Vespidae). *Biol. Rev.* 46, 483–528. <https://doi.org/10.1111/j.1469-185X.1971.tb01054.x>.
- Ríos V., M., Barrera-Medina, R., Contreras F., J.M., Ríos V., M., Barrera-Medina, R., Contreras F., J.M., 2020. Primer reporte del género *Vespa* Linnaeus (Hymenoptera: Vespidae: Vespinae) en Chile. *Rev. Chil. Entomol.* 46, 237–242. <https://doi.org/10.35249/rche.46.2.20.14>.
- Rojas-Nossa, S.V., Novoa, N., Serrano, A., Calviño-Cancela, M., 2018. Performance of baited traps used as control tools for the invasive hornet *Vespa velutina* and their impact on non-target insects. *Apidologie* 49, 872–885. <https://doi.org/10.1007/s13592-018-0612-0>.
- Rojas-Nossa, S.V., Mato, S., Feijoo, P., Lagoa, A., Garrido, J., 2024. Comparison of effectiveness and selectiveness of baited traps for the capture of the invasive hornet *Vespa velutina*. *Animals* 14, 129. <https://doi.org/10.3390/ani14010129>.
- Rose, E.A.F., Harris, R.J., Glare, T.R., 1999. Possible pathogens of social wasps (Hymenoptera: Vespidae) and their potential as biological control agents. *NZ J. Zool.* 26, 179–190. <https://doi.org/10.1080/03014223.1999.9518188>.
- Ruschioni, S., Lorenzini, M., Fontana, P., 2023. *Vespa orientalis* (Hymenoptera; Vespidae), the first record in Marche region. <https://doi.org/10.5281/zenodo.10083019>.
- Sadd, B.M., Schmid-Hempel, P., 2006. Insect immunity shows specificity in protection upon secondary pathogen exposure. *Curr. Biol.* 16, 1206–1210. <https://doi.org/10.1016/j.cub.2006.04.047>.
- Sánchez, O., Castro, L., Fueyo, Á., Borrell, Y.J., Arias, A., 2024. Early alarm on the first occurrence of the southern Giant hornet *Vespa soror* du Buysson, 1905 (Vespidae) in Europe. *Ecol. Evol.* 14, e70502. <https://doi.org/10.1002/ee3.70502>.
- Senanayake, I.C., Rossi, W., Leonardi, M., Weir, A., McHugh, M., Rajeshkumar, K.C., Verma, R.K., Karunarathna, S.C., Tibpromma, S., Ashtekar, N., Ashtamoorthy, S.K., Raveendran, S., Kour, G., Singh, A., De la Peña-Lastra, S., Mateos, A., Kolařík, M., Antonín, V., Ševčíková, H., Esteve-Raventós, F., Larsson, E., Pancorbo, F., Moreno, G., Altés, A., Turégano, Y., Du, T.-Y., Lu, L., Li, Q.-R., Kang, J.-C.,

- Gunaseelan, S., Kezo, K., Kaliyaperumal, M., Fu, J., Samarakoon, M.C., Gafforov, Y., Teshaboeva, S., Kunjan, P.C., Chamaparambath, A., Flakus, A., Etayo, J., Rodriguez-Flakus, P., Zhurbenko, M.P., de Silva, N.I., Tennakoon, D.S., Latha, K.P.D., Manimohan, P., Raj, K.N.A., Calabon, M.S., Ahmadpour, A., Heidarian, Z., Alavi, Z., Alavi, F., Ghosta, Y., Azizi, R., Luo, M., Zhao, M.-P., Kularathnage, N.D., Hua, L., Yang, Y.-H., Liao, C.-F., Zhao, H.-J., Lestari, A.S., Jayasiri, S.C., Yu, F.-M., Lei, L., Liu, J.-W., Karimi, O., Tang, S.-M., Sun, Y.-R., Wang, Y., Zeng, M., Htet, Z.H., Linaldeddu, B.T., Alves, A., Phillips, A.J.L., Bregant, C., Montecchio, L., De Kesel, A., Hustad, V.P., Miller, A.N., Fedosova, A.G., Kucera, V., Raza, M., Hussain, M., Chen, Y.-P., Thiagaraja, V., Gomdola, D., Rathnayaka, A.R., Dissanayake, A.J., Suwannarach, N., Hongsanan, S., Maharachchikumbura, S.S.N., Dissanayake, L.S., Wijayawardene, N.N., Phookamsak, R., Lumyong, S., Jones, E.B.G., Yapa, N., Wanasinghe, D.N., Xie, N., Doilom, M., Manawasinghe, I.S., Liu, J.-K., Zhao, Q., Xu, B., Hyde, K.D., Song, J., 2023. Fungal diversity notes 1611–1716: taxonomic and phylogenetic contributions on fungal genera and species emphasis in South China. *Fungal Divers.* 122, 161–403. <https://doi.org/10.1007/s13225-023-00523-6>.
- Simmons, D.R., Kepler, R.M., Rehner, S.A., Groden, E., 2015. Phylogeny of *Hirsutiella* species (Ophiocordycipitaceae) from the USA: remedying the paucity of *Hirsutiella* sequence data. *IMA Fungus* 6, 345–356. <https://doi.org/10.5598/imafungus.2015.06.02.06>.
- Smith-Pardo, A.H., Altamiranda-Saavedra, M., Polly, P.D., 2024. The oriental hornet, *Vespa orientalis* Linnaeus, 1771 (Hymenoptera, Vespidae): diagnosis, potential distribution, and geometric morphometrics across its natural distribution range. *Front. Insect Sci.* 4. <https://doi.org/10.3389/finsc.2024.1384598>.
- Stefanini, I., 2018. Yeast-insect associations: it takes guts. *Yeast* 35, 315–330. <https://doi.org/10.1002/yea.3309>.
- Stefanini, I., Dapporto, L., Legras, J.-L., Calabretta, A., Di Paola, M., De Filippo, C., Viola, R., Capretti, P., Polsinelli, M., Turillazzi, S., Cavalieri, D., 2012. Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. *Proc. Natl. Acad. Sci.* 109, 13398–13403. <https://doi.org/10.1073/pnas.1208362109>.
- Suh, S.-O., Nguyen, N.H., Blackwell, M., 2008. Yeasts isolated from plant-associated beetles and other insects: seven novel *Candida* species near *Candida albicans*. *FEMS Yeast Res.* 8, 88–102. <https://doi.org/10.1111/j.1567-1364.2007.00320.x>.
- Sweelam, M.E., Abdelaal, A.A., Khattaby, A.M., Mettwaly, Y.A., 2019. Integrated management of *Vespa orientalis* in honeybee colonies at Elsharkia Governorate. *Menoufia J. Plant Prot.* <https://doi.org/10.21608/mjapam.2019.122988>.
- Tan, K., Hu, Z., Chen, W., Wang, Z., Wang, Y., Nieh, J.C., 2013. Fearful foragers: honey bees tune Colony and individual foraging to multi-predator presence and food quality. *PLoS One* 8, e75841. <https://doi.org/10.1371/journal.pone.0075841>.
- Temreshev, I.I., 2018. On the expansion of the areas of *Vespa orientalis* and *Polistes wattii* (Hymenoptera: Vespidae) on the territory of the Republic of Kazakhstan. *Acta Biol. Sib.* 4, 38. <https://doi.org/10.14258/abs.v4i1.3915>.
- Turchi, L., Derijard, B., 2018. Options for the biological and physical control of *Vespa velutina nigrithorax* (Hym.: Vespidae) in Europe: a review. *J. Appl. Entomol.* 142, 553–562. <https://doi.org/10.1111/jen.12515>.
- Turillazzi, S., Meriggi, N., Cavalieri, D., 2023. Mutualistic relationships between microorganisms and eusocial wasps (Hymenoptera, Vespidae). *Microorganisms* 11, 1340. <https://doi.org/10.3390/microorganisms11051340>.
- Valero-Jiménez, C.A., Faino, L., Spring in't Veld, D., Smit, S., Zwaan, B.J., van Kan, J.A.L., 2016a. Comparative genomics of *Beauveria bassiana*: uncovering signatures of virulence against mosquitoes. *BMC Genomics* 17, 986. <https://doi.org/10.1186/s12864-016-3339-1>.
- Valero-Jiménez, C.A., Wiegers, H., Zwaan, B.J., Koenraadt, C.J.M., van Kan, J.A.L., 2016b. Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*. *J. Invertebr. Pathol.* 133, 41–49. <https://doi.org/10.1016/j.jip.2015.11.011>.
- Van Itterbeeck, J., Feng, Y., Zhao, M., Wang, C., Tan, K., Saga, T., Nonaka, K., Jung, C., 2021. Rearing techniques for hornets with emphasis on *Vespa velutina* (Hymenoptera: Vespidae): a review. *J. Asia Pac. Entomol.* 24, 103–117. <https://doi.org/10.1016/j.jaspen.2021.03.009>.
- Veen, K.H., 1966. Oral infection of second-instar nymphs of *Schistocerca gregaria* by *Metarrhizium anisopliae*. *J. Invertebr. Pathol.* 8, 254–256. [https://doi.org/10.1016/0022-2011\(66\)90138-8](https://doi.org/10.1016/0022-2011(66)90138-8).
- Werenkraut, V., Arbetman, M.P., Fergnani, P.N., 2022. The oriental hornet (*Vespa orientalis* L.): a threat to the Americas? *Neotrop. Entomol.* 51, 330–338. <https://doi.org/10.1007/s13744-021-00929-4>.
- Wilson, T.M., Takahashi, J., Spichiger, S.-E., Kim, I., van Westendorp, P., 2020. First reports of *Vespa mandarinia* (Hymenoptera: Vespidae) in North America represent two separate maternal lineages in Washington state, United States, and British Columbia, Canada. *Ann. Entomol. Soc. Am.* 113, 468–472. <https://doi.org/10.1093/aesa/saaa024>.
- Wrońska, A.K., Kaczmarek, A., Boguś, M.I., Kuna, A., 2023. Lipids as a key element of insect defense systems. *Front. Genet.* 14, 1183659. <https://doi.org/10.3389/fgene.2023.1183659>.
- Yanagita, T., Iwashita, Y., 1987. Histological observation of larvae of the silkworm, *Bombyx mori*, orally infected with *Beauveria bassiana*. *J. Seric. Sci. Jpn.* 56, 285–291. <https://doi.org/10.11416/kontyushigen1930.56.285>.
- Zachi, M., Ruicănescu, A., 2021. *Vespa orientalis*, a new alien species in Romania. *Trav. Muséum Natl. D'Histoire Nat. "Grigore Antipa"* 64, 67–72. <https://doi.org/10.3897/travaux.64.e61954>.
- Zucca, P., Granato, A., Mutinelli, F., Schiavon, E., Bordin, F., Dimech, M., Balbo, R.A., Mifsud, D., Dondi, M., Cipolat-Gotet, C., Rossmann, M.C., Ocepek, M.P., Scaravelli, D., Palei, M., Zinzula, L., Spanjol, K., 2024. The oriental hornet (*Vespa orientalis*) as a potential vector of honey bee's pathogens and a threat for public health in north-East Italy. *Vet. Med. Sci.* 10, e1310. <https://doi.org/10.1002/vms3.1310>.