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Microbiota and pathogens in an invasive bee: *Megachile sculpturalis* from native and invaded regions

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Abstract

The present study aimed to characterise the bacterial, fungal and parasite gut community of the invasive bee *Megachile sculpturalis* sampled from native (Japan) and invaded (USA and France) regions via 16S rRNA and ITS2 amplicon sequencing and PCR detection of bee microparasites. The bacterial and fungal gut microbiota communities in bees from invaded regions were highly similar and differed strongly from those obtained in Japan. Core amplicon sequence variants (ASVs) within each population represented environmental micro-organisms commonly present in bee-associated niches that likely provide beneficial functions to their host. Although the overall bacterial and fungal communities of the invasive *M. sculpturalis* in France and the co-foraging native bees *Anthidium florentinum* and *Halictus scabiosae*, were significantly different, five out of eight core ASVs were shared suggesting common environmental sources and potential transmission. None of the 46 *M. sculpturalis* bees analysed harboured known bee pathogens, while microparasite infections were common in *A. florentinum*, and rare in *H. scabiosae*. A common shift in the gut microbiota of *M. sculpturalis* in invaded regions as a response to changed environmental conditions, or a founder effect coupled to population re-establishment in the invaded regions may explain the observed microbial community profiles and the absence of parasites. While the role of pathogen pressure in shaping biological invasions is still debated, the absence of natural enemies may contribute to the invasion success of *M. sculpturalis*.

KEYWORDS
Anthidium florentinum, gut microbiota, *Halictus scabiosae*, *Megachile sculpturalis*, parasites

INTRODUCTION

Increasing occurrence of alien species beyond their native range is a well-known effect of globalisation (Seebens et al., 2017). International trading networks and expanding urbanisation are drivers involved in the introduction and the spread of invasive species into novel areas. The introduction of bee species occurred both intentionally, for their role as managed pollinators and therefore economic purposes (Arretz & Macfarlane, 1986; Gurr, 1972; Pitts-Singer & Cane, 2011; Torchio et al., 1987), and accidentally through trading points (Bortolotti et al., 2018; Mangum & Brooks, 1997; Vereecken & Barbier, 2009). In 2016, it was estimated that globally 80 bee species

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have been introduced into a foreign habitat (Russo, 2016). Bees are generally perceived as beneficial because of their pollination services. Some introduced bees though are now widely recognised to have negative impacts on the ecosystem that include the competition with native species for nesting sites and floral resources, and the introduction of foreign pathogens (Goulson, 2003; Russo, 2016).

Among the introduced bees, the family Megachilidae, and more specifically, the genus *Megachile* is best represented (Russo, 2016). *Megachile sculpturalis* Smith, 1853 is a large cavity-nesting solitary bee recognised as an invasive species in the USA and with indications to be invasive in Europe. *Megachile sculpturalis* is native to East-Asia (Japan, China, South-Korea and Taiwan) (Iwata, 1933) and was first recorded outside of its natural geographical range in North Carolina (Eastern USA) in 1994 (Mangum & Brooks, 1997). In Europe, *M. sculpturalis* was first observed near Marseille (Southern France) in 2008 (Vereecken & Barbier, 2009). In both cases, *M. sculpturalis* was accidentally introduced, most probably through maritime trading as a stowaway on ships, likely as overwintering larvae in wood (le Féon et al., 2018; Meurisse et al., 2019; Quaranta et al., 2014). Since the first observation in 1994, its range rapidly expanded across all states along the eastern coast to mid USA and Canada (Mangum & Sumner, 2003; Paiero & Buck, 2003; Parys et al., 2015). *Megachile sculpturalis* also successfully expanded its distribution on the European continent. From France it migrated into Italy (Quaranta et al., 2014) and Switzerland (Amiet, 2012), and continued further into Germany (Westrich, 2015), Hungary (Kovács, 2015), Slovenia (Gogala & Zadravec, 2018), Spain (Ortiz-Sánchez et al., 2018), Serbia (Ćetković & Plećaš, 2017), Austria, Liechtenstein, Croatia, Bosnia Herzegovina (Dubaić et al., 2021), and the Crimean Peninsula (Ivanov & Fateryga, 2019). The range expansion of *M. sculpturalis* occurred remarkably fast and two dispersal mechanisms were identified: first, by diffusion dispersal characterised by active small-scale expansion and secondly, through jump dispersal to passively overcome long distances, likely using a transportation vector (Lanner et al., 2020). Genetic profiling of *M. sculpturalis* populations in Middle Europe recently identified two genetic clusters and suggested multiple invasion events on the European continent (Lanner et al., 2021). Its spread over Europe seems to be in an early stage and model projections suggested that large areas are still at risk of invasion (Lanner et al., 2022).

Megachile sculpturalis evicts pre-existing nests of other native cavity-nesting bees, such as *Xylocopa* spp. and *Osmia* spp. (Lanner et al., 2020; Laport & Minckley, 2012; Le Féon et al., 2018; Roulston & Malfi, 2012), and frequently occupies bee hotels set up in urban areas to promote wild bee populations. *M. sculpturalis* brood occupied the outer positions in tunnels cohabitated by *Osmia cornuta* brood, indicating that *O. cornuta* emergence in spring can be indirectly blocked by the summer-emerging *M. sculpturalis* (Díaz et al., 2021). These findings suggested that native cavity-nesting bees experience the primary negative impacts of *M. sculpturalis* invasion, and its presence was negatively correlated with the emergence of native wild bees (Geslin et al., 2020). An impact that is less visible is the introduction of diseases by exotic species which might even facilitate its invasion (Dunn, 2009; Parker et al., 2013). Spillover of foreign pathogens and parasites to native bee species has been recorded for exotic bumble bees. Various *Bombus* species have been introduced deliberately all over the globe in the 20th century for crop pollination as a result of commercial rearing (Arretz & Macfarlane, 1986; Goulson, 2010; Gurr, 1972). The introduced bumble bees harboured pathogens that could spill over to native bee species and cause population declines (Arbetman et al., 2013; Goulson, 2010; Meeus et al., 2011). Besides parasite spillover, escape from natural enemies when entering new regions can increase the invasion success of the exotic species (Colautti et al., 2004). Recently, the occurrence of macroparasites in *M. sculpturalis* brood cells has been reported (Díaz et al., 2021), but the presence of microparasites remains unstudied.

The successful invasion of novel geographical regions by *M. sculpturalis* can be attributed to its intrinsic characteristics such as its generalist diet, large body size and cavity-nesting behaviour (Le Féon et al., 2018), but anthropogenic factors such as human population densities and distance to roads have facilitated its expansion as well (Lanner et al., 2022). The mechanisms underlying adaptation and plasticity of this species to these novel environments remain unexplored. Researchers are increasingly aware of the importance of the microbiome component within the 'holobiont' concept (Maebe et al., 2021) to understand host health and nutrition (Engel & Moran, 2013; Miller et al., 2021). Microbes experience genetic evolution at a higher pace than their bee hosts and can be shared between pollinators via flowers (Gibson et al., 2018; Keller et al., 2021). They therefore represent a highly dynamic adaptive potential, both plastic and selective, to environmental pressures that challenge its host health and survival (Keller et al., 2021).

In the present study, we characterised the bacterial, fungal and parasite community in the gut of *M. sculpturalis* to improve our understanding of the mechanism of its invasion success and to gain more insight into its adaptations to newly invaded regions. We analysed samples from both native (Japan) and invaded habitats (USA and France) to determine if their microbial communities shifted during migration to novel geographical regions. Furthermore, we determined the microbial communities of two native co-foraging bee species, that is, *Anthidium florentinum* and *Halictus scabiosae*, to assess sharing of gut microbes between native and invasive species. The former represented a species that is phylogenetically closely related to *M. sculpturalis* and that shares a similar nesting habit, the latter represented a bee species that is phylogenetically distant and with a different nesting habit.

RESULTS

Library statistics and gut microbiota community composition

The gut microbial communities of 25 *M. sculpturalis*, 15 *A. florentinum* and 9 *H. scabiosae* bees were examined through 16S rRNA and ITS2 amplicon sequencing (Table S1). A total of 1,882,732 and 2,997,042

demultiplexed paired-end reads of the 16S rRNA V3-V4 and ITS2 region were obtained, respectively. A total of 1,550,964 16S rRNA V3-V4 and 752,885 ITS2 high quality reads were retained after DADA2 analysis. 16S rRNA reads clustered into 1577 ASVs and ITS2 reads clustered into 641 ASVs. Rarefaction curves indicated that a sufficient sampling depth was reached for the bacterial community, whereas the ITS2 sampling was near saturation for only some samples (Figure S1). Data cleaning retained 1499 bacterial and 255 fungal ASVs. *M. sculpturalis* sample MS08 and *H. scabiosae* sample HS03 were excluded from the fungal analysis as no fungal ITS2 reads were detected in these samples.

The most abundant ASV in the 16S rRNA dataset was assigned to *Brevibacillus borstelensis* which was detected in all samples. ASVs of the genera *Brevibacillus*, *Apilactobacillus*, *Acinetobacter*, *Weissella* and *Rosenbergiella* were the most abundant, but their abundance varied between bee species. Four of the 20 most abundant ASVs could not be classified at the genus level; at family level three could be assigned to Enterobacteriaceae and one was assigned at order level to Corynebacteriales. The majority of ITS2 reads were attributed to an ASV that could not be classified beyond the order Saccharomycetales. Other abundant genera in the ITS2 dataset included *Metschnikowia*, *Starmerella* and *Candida*. The bacterial and fungal community composition based on the 20 most relatively abundant ASVs is shown in Figure 1.

Megachile sculpturalis gut microbial community dissimilarity in native and invasive bees

The *M. sculpturalis* microbiota composition was determined for bees sampled from their native region (Japan) and from two invaded regions (USA and France). PERMANOVA analysis indicated that bacterial and fungal communities of *M. sculpturalis* differed significantly between sampled locations ($R^2 = 0.62$, $p < 0.001$ and $R^2 = 0.24$, $p < 0.05$, respectively). Analysis of group dispersions showed that the bacterial data were heterogeneously dispersed ($p < 0.001$), reducing the explanatory power of the PERMANOVA test, whereas the fungal data were homogeneously dispersed ($p = 0.158$).

Pairwise PERMANOVA tests demonstrated that the bacterial communities of *M. sculpturalis* at the two locations in Japan were not significantly different ($p_{adj} = 0.24$). Likewise, the bacterial communities in bees from the two invaded regions were not significantly different either ($p_{adj} = 0.26$). When comparing the bacterial community of the native Japanese bees with the invasive bees, however, differences in bacterial communities were observed with bees from Marseille ($p_{adj} < 0.001$) and from New York ($p_{adj} < 0.01$). Pairwise PERMANOVA tests did not reveal significant differences for the fungal communities of bees from different locations (Table S2). Principal Coordinates Analysis (PCoA) ordination confirmed that *M. sculpturalis* bacterial communities of native and invasive bees clustered separately (Figure 2a), whereas no clear clustering by location was observed for the fungal community (Figure 2b).

Bacterial and fungal ASVs that were differentially abundant in the three sampled countries were identified using DESeq2 by estimating the log2 fold difference. Pairwise comparisons of the bee microbiota between these locations, revealed discriminating ASVs for each location (Tables S3, S4).

Gut microbiota community of *M. sculpturalis* in Japan

One bacterial (*Rosenbergiella*) and five fungal ASVs (Saccharomycetales incertae sedis [two ASVs], *Moniliella*, and *Metschnikowia* [two ASVs]) were identified as core gut microbes in Japanese *M. sculpturalis* samples (Figure 3). *Moniliella* and *Kosakonia* ASVs were more abundant in Japanese *M. sculpturalis* than in those of New York, while *Moniliella* and both *Metschnikowia* ASVs were more abundant in Japanese *M. sculpturalis* than in those of Marseille (Tables S3, S4).

Samples of Okayama and Ibaraki had the lowest bacterial richness among all *M. sculpturalis* samples (Figure S2A), while Ibaraki samples had the highest fungal richness (Figure S2C). Although the Shannon diversity was overall significantly different between sampling locations (Figures S2B, S2D), no significant pairwise differences were found (Table S5). Japanese samples were dominated by proteobacterial taxa, with *Rosenbergiella* as the most abundant genus. Considerable inter-individual variation was observed, especially in Ibaraki samples. At the latter location, the four analysed samples were each dominated by reads of a different proteobacterial genus (Figure 1a). Okayama samples were almost exclusively dominated by the fungal taxon Saccharomycetales incertae sedis (Figure 1b), whereas Ibaraki samples showed a higher richness (Figure S2C) with 3 to 4 dominating fungal genera in every sample.

Gut microbiota community of *M. sculpturalis* in invaded regions

The bacterial and fungal gut communities of *M. sculpturalis* were similar at both sampling sites in Marseille ($R^2 = 0.06$, $p = 0.61$ and $R^2 = 0.08$, $p = 0.35$, respectively) and for both male and female bees ($R^2 = 0.05$, $p = 0.89$ and $R^2 = 0.13$, $p = 0.36$, respectively). Additionally, the bacterial and fungal gut communities of *M. sculpturalis* samples in New York were highly similar to those in Marseille ($p_{adj} = 0.26$ and $p_{adj} = 0.16$, respectively, Table S2). DESeq2 analysis revealed three bacterial and one fungal ASV that were differentially abundant between the two invaded areas (Tables S3, S4).

Megachile sculpturalis gut samples from these invaded regions showed a consistent bacterial composition that was dominated by *Brevibacillus*, followed by Enterobacteriaceae, *Bacillus*, *Acidocella* and *Lactococcus* (Figure 1a). The bacterial community in Marseille samples showed occasional occurrences of the genera *Carnimonas*, *Weissella*, *Apilactobacillus* and *Acinetobacter* among the dominant ASVs. The fungal composition was almost exclusively composed of a Saccharomycetales incertae sedis taxon (Figure 1b). In New York samples, there was also a dominant occurrence of *Candida*, whereas in Marseille samples, some individuals showed more diversity with the occurrence of *Alternaria*, *Metschnikowia* or *Starmerella*. One sample from Marseille

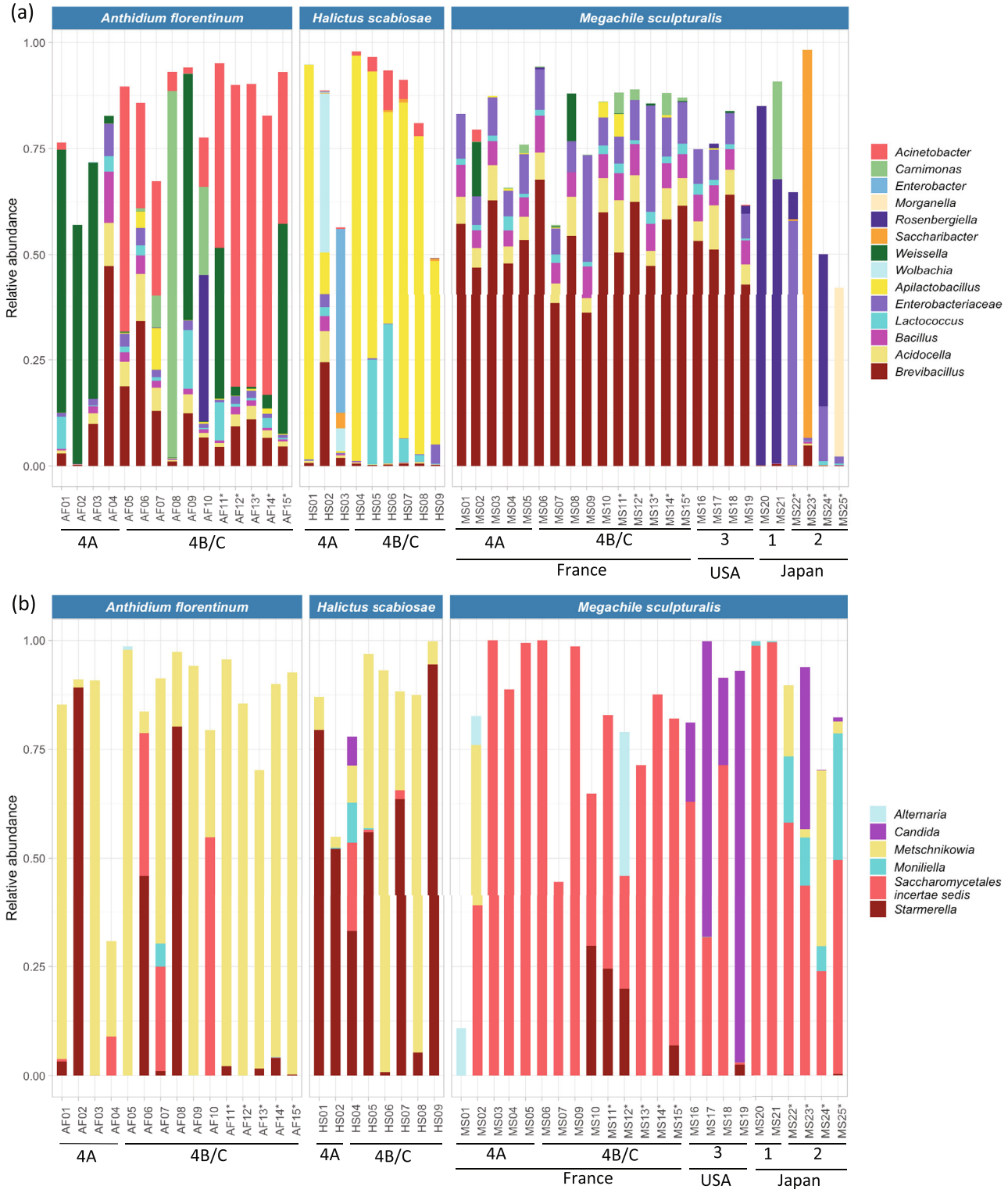

FIGURE 1 Genus-level bar plots showing the relative abundance of the (a) bacterial and (b) fungal ASVs of the samples grouped by bee species and sampling location. Only the 20 most relative abundant ASVs are shown. Sampling locations are Mukuchi Island, Okayama (1) and Tsukuba City, Ibaraki (2) in Japan; New York City (3), USA; and Parc Borély (4A), and Parc Bonneveine and Parc Bortoli grouped as one sampling site (4B/C), Marseille, France. Male bees are annotated with *.

(MS01) had a highly distinct fungal composition dominated by *Candida* and *Alternaria*. Seven bacterial ASVs (assigned to *Brevibacillus borstelensis*, *Brevibacillus*, Enterobacteriaceae [two ASVs], *Bacillus*, *Acidocella facilis* and *Lactococcus*) and 2 fungal ASVs (assigned to Saccharamycetales incertae sedis and *Candida*) were identified as core gut microbes in *M. sculpturalis* in New York (Figure 3). *Megachile sculpturalis* from

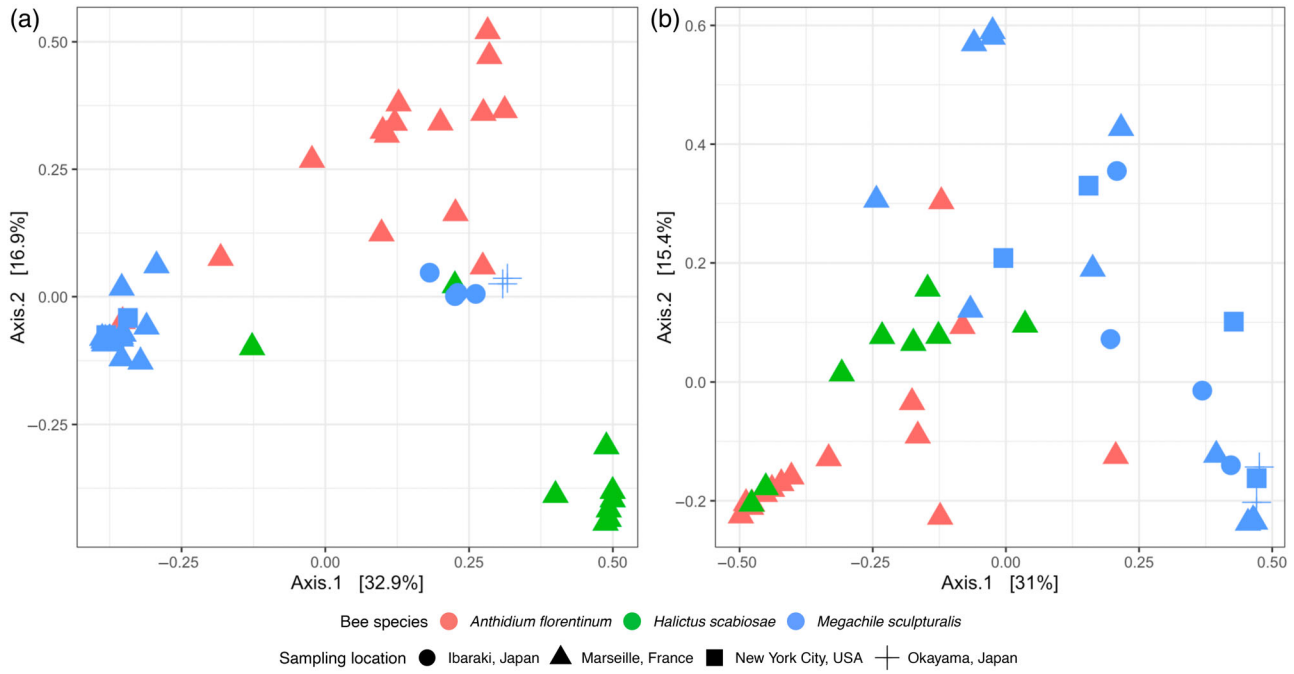

FIGURE 2 Principal Coordinates Analysis ordination based on Bray–Curtis dissimilarity matrices of the (a) bacterial and (b) fungal communities grouped by bee species and sampling location.

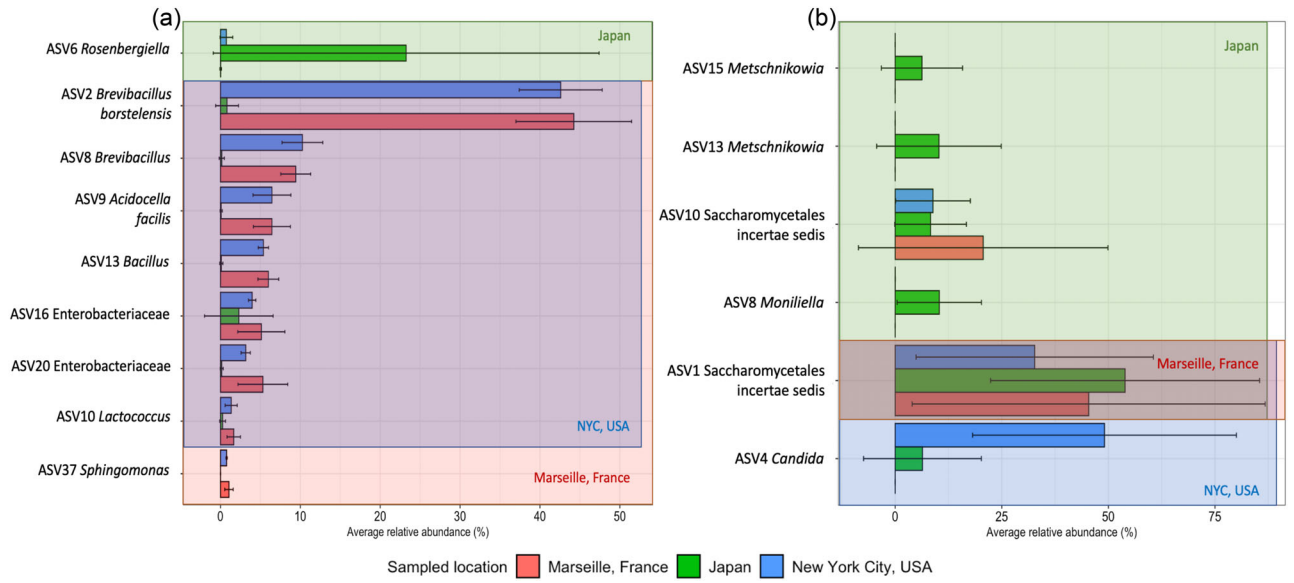

FIGURE 3 Relative abundance of core (a) bacterial and (b) fungal ASVs of *M. sculpturalis* in Marseille, France (red rectangle); the two Japanese locations Mukuchi Island, Okayama and Tsukuba City, Ibaraki (green rectangle); and New York City, USA (blue rectangle). The average relative abundance of each core ASV is shown in coloured bars corresponding to each of the sampling locations. Error bars represent standard deviation.

Marseille had one additional *Sphingomonas* ASV as part of its bacterial core (Figure 3a) but contained only one ASV (Saccharomycetales incertae sedis) in its fungal core (Figure 3b).

Finally, the bacterial gut community of *M. sculpturalis* in invaded regions differed significantly from that in Japan ($p_{adj} < 0.01$, Table S2), whereas the fungal community did not. The bacterial community was characterised by four (New York) and eight (Marseille) bacterial ASVs that were differentially abundant compared to the Japanese locations examined (Table S3).

Gut microbiota community of native co-foraging bees in Marseille

The bacterial gut community of *A. florentinum* was dominated by *Acinetobacter*, *Weissella*, *Brevibacillus* and *Carnimonas*, whereas the fungal community was mainly characterised by *Metschnikowia* with some occurrences of *Starmerella* and Saccharomycetales incertae sedis (Figure 1). The bacterial gut community of *A. florentinum* differed

significantly between male and female bees ($R^2 = 0.20$, $p < 0.05$) and between sampling sites in Marseille ($R^2 = 0.23$, $p < 0.01$), whereas the fungal community did not. Nine bacterial and 2 fungal ASVs were differentially abundant in *A. florentinum* between sampling sites (Tables S3, S4), and one fungal ASV was differentially abundant between male and female *A. florentinum* bees (Table S4). The bacterial gut community of *H. scabiosae* was dominated by *Apilactobacillus*, whereas the fungal community was mainly characterised by *Starmerella* and *Metschnikowia* (Figure 1). The bacterial and fungal gut communities of *H. scabiosae* differed significantly between sampling sites in Marseille ($R^2 = 0.31$, $p < 0.05$ and $R^2 = 34$, $p < 0.05$, respectively). Five bacterial and 9 fungal ASVs were differentially abundant between the two sites (Tables S3, S4).

The core microbiota of *A. florentinum* was composed of 7 bacterial ASVs (assigned to *Brevibacillus borstelensis*, *Acinetobacter nectaris*, *Weissella*, *Brevibacillus*, *Acidocella facilis*, *Bacillus* and *Acinetobacter*) and 6 fungal ASVs (assigned to *Metschnikowia gruessii* [4 ASVs] and *Metschnikowia reukaufii* [2 ASVs]); that of *H. scabiosae* was composed of 2 bacterial (*Apilactobacillus* and *Lactococcus*) and 4 fungal ASVs (*Metschnikowia gruessii*, *Metschnikowia reukaufii* [2 ASVs] and *Starmerella*) (Figure 4). Although some core ASVs were shared between different hosts (Figure 4), several differentially abundant ASVs for each pair of hosts were identified (Tables S3, S4). PERMANOVA analysis and clustering in the PCoA ordination of Bray–Curtis distances (Figure 2) revealed a significant difference in bacterial and fungal community between the three bee species from Marseille ($R^2 = 0.496$, $p < 0.001$ and $R^2 = 0.26$, $p < 0.001$, respectively). Pairwise PERMANOVA tests further demonstrated that the microbial communities were significantly different between each of the three species ($p_{adj} < 0.01$ for all group pairings, Table S2). Furthermore, a significant dispersion was detected between bacterial community profiles ($p < 0.001$) but not for the fungal community profiles ($p = 0.89$), and there was a significant difference in alpha diversity estimates between the three hosts (Figure S3). *Halictus scabiosae* was characterised by the lowest bacterial richness and Shannon diversity, while *A. florentinum* and *M. sculpturalis* displayed similar richness but different Shannon diversity indices (Table S5). Fungal alpha diversity estimates were lowest for *M. sculpturalis* and similar between *A. florentinum* and *H. scabiosae* (Table S5).

Parasite prevalence

Parasite DNA was detected in none of the 46 *M. sculpturalis* specimens and in only one out of 22 *H. scabiosae* specimens examined. The latter parasite was identified as a *Crithidia* sp. using genus-specific primers (Table S6, set 2B), while 18S rRNA sequence analysis revealed 97.41% sequence identity towards *Crithidia*, *Wallaceina*, *Leptomonas* and *Blastocrithidia* sequences (Table S7). *Anthidium florentinum* samples had an overall parasite prevalence of 25% (7 out of 28 bees). Parasites in two of these bees were identified as *Crithidia* sp. and exhibited 97.09% and 97.0% 18S rRNA sequence identity towards *Crithidia*, *Wallaceina*, *Leptomonas* and *Blastocrithidia* sequences (Table S7 and Figure S4). One *A. florentinum* bee tested positive with *Nosema*-specific primers; its amplicon sequence yielded 99.61% 18S rRNA sequence identity towards sequences of *Nosema granulosis* and an unspecified *Nosema* sp. (Table S7 and Figure S5). Finally, *Apicystis* parasites were identified as *Apicystis cryptica* (3 bees) and *Apicystis bombi* (1 bee) based on an earlier re-evaluation of publicly available *Apicystis* sequences [as per Supplementary file 1 in Schoonvaere et al. (2020)].

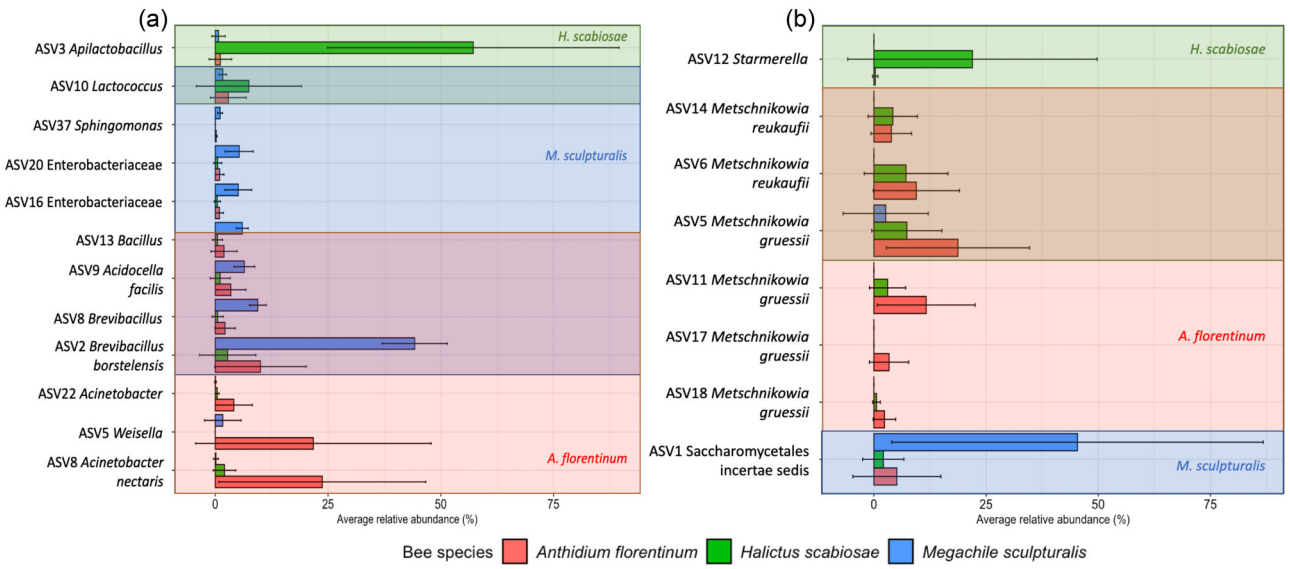

FIGURE 4 Relative abundance of core (a) bacterial and (b) fungal ASVs of *A. florentinum* (red rectangle), *H. scabiosae* (green rectangle) and *M. sculpturalis* (blue rectangle) sampled in Marseille, France. The average relative abundance of each core ASV is shown in coloured bars corresponding to each of the bee species. Error bars represent standard deviation.

DISCUSSION

In the present study, we analysed the gut microbial communities of *M. sculpturalis*, an invasive bee in Europe and North America. While its invasion success has been attributed to both intrinsic as well as exogenous, Anthropocene-specific factors (Lanner et al., 2022; Le Féon et al., 2018), the role of its gut microbiota and its capacity to host parasites remained unstudied. We therefore examined the gut microbial composition of *M. sculpturalis* from native (Japan) and invaded regions (USA and France) and compared it with that of *A. florentinum* and *H. scabiosae*, two co-foraging bees captured in France. While the number of samples from Japan and the USA was limited, the microbial composition of *M. sculpturalis* differed significantly between native and invaded locations, and was distinct from that of co-foraging bees captured in France. Moreover, we did not detect parasites in any of the populations, invaded or not. Overall, the majority of the dominant gut microorganisms were primarily known as environmental microbes, some of which were previously reported in association with bees.

Bacterial core ASVs of *M. sculpturalis*

The overall difference in gut microbial composition of native and invasive *M. sculpturalis* bees was reflected in the absence of shared bacterial core ASVs (Figure 3a). Only one core ASV was identified in the Japanese *M. sculpturalis* population. It was taxonomically assigned to *Rosenbergiella* with 100% sequence identity to both *Rosenbergiella epipactidis* and *Rosenbergiella nectarea* 16S rRNA sequences, and was the most dominant ASV in the native population. *Rosenbergiella* species are commonly associated with floral nectar (Halpern et al., 2013; Lenaerts et al., 2014) and pollen (Ambika Manirajan et al., 2016) which likely explains their occurrence in bee guts. The *R. nectarea* genome is tailored to a life in a plant environment and includes genes that encode pectinase activity to attack cell or pollen walls, the production of bioactive metabolites with antimicrobial activity and the production of volatile compounds that may impact flower visitation by nectar consumers (Laviad-Shitrit et al., 2020), all highlighting a potential beneficial role within the bee host.

The bacterial gut community of *M. sculpturalis* was highly similar in both invaded regions examined. Seven core ASVs taxonomically assigned to *Brevibacillus borstelensis*, *Brevibacillus*, *Acidocella facilis*, *Lactococcus*, *Bacillus* and Enterobacteriaceae (2 ASVs) were shared between the two populations (Figure 3a). *Brevibacillus* species are widely distributed in the environment (Yang & Yousef, 2018) and are associated with several insects (An et al., 2020; Ayyasamy et al., 2021). In particular, *Brevibacillus laterosporus* is a gut symbiont of honey bees and bumble bees but is a pathogen in other insects (Marche et al., 2016). *B. borstelensis* has industrial applications such as plastic degradation (Khalil et al., 2018) but was not reported in insects before. *Brevibacillus* has proven to be a rich source of antimicrobial peptides (Yang & Yousef, 2018) and might thus provide beneficial functions to its insect hosts. *Acidocella* bacteria are acidophilic organisms linked to extreme environments (Kishimoto et al., 1995) but have not been reported in insect guts before. *Lactococcus* was the only lactic acid bacterium identified as a core member of *M. sculpturalis*. Lactococci occur in many habitats, ranging from plant-associated to dairy environments, and have been reported as gut symbionts in several insect species (Cini et al., 2020; Lombogia et al., 2020; Muhammad et al., 2017). Environmental lactococci digest plant polymers and ferment carbohydrates into lactic acid (Passerini et al., 2013; Wels et al., 2019). The former function enables the host to acquire otherwise inaccessible nutrients, while the latter may help protect against pathogens via gut acidification. *Bacillus* spp. and Enterobacteriaceae occur in various environmental sources and have been detected in the gut and nest contents of other megachilid bees (Inglis et al., 1993; Keller et al., 2013; Voulgari-Kokota et al., 2018; Voulgari-Kokota et al., 2019). Some bacilli are known as insect pathogens (Stahly et al., 2006) while others exhibit beneficial functions such as the degradation of complex polysaccharides and aromatic compounds (König, 2006). Enterobacteriaceae can exhibit positive functions in insect hosts such as resistance to *Plasmodium* in mosquitoes (Cirimotich et al., 1979) and enhance survival, sexual fitness, and nitrogen fixation in the Mediterranean fruit fly (Behar et al., 2008). One additional core ASV in *M. sculpturalis* from France was identified as *Sphingomonas*, a ubiquitous environmental bacterium previously detected in honey bees and solitary bees (Anjum et al., 2018; Graystock et al., 2017) but with unknown function.

Fungal core ASVs of *M. sculpturalis*

The fungal core ASVs also differed between native and invaded regions although one Saccharomycetales incertae sedis ASV was shared between the three populations (Figure 3b). Fungal core ASVs of Japanese *M. sculpturalis* were classified as Saccharomycetales incertae sedis (2 ASVs), *Moniliella* and *Metschnikowia* (2 ASVs). The former is a taxon containing yeast genera with unknown phylogenetic relationships and could thus not be classified beyond the order level (Kurtzman et al., 2011). The two ASVs classified within this group may therefore each represent multiple yeast species with a similar ITS2 sequence but showed the highest sequence similarity to *Starmerella kuoi* (95.69% and 95.29%, respectively). Fewer fungal core ASVs were identified in invaded regions, in accordance with a lower fungal richness. Saccharomycetales incertae sedis and *Candida* ASVs were core ASVs in *M. sculpturalis* in the USA, while the former was the only core ASV of *M. sculpturalis* in France (Figure 3b). Flowers and flower-visiting insects have been identified as common habitats for *Starmerella* (da Costa Neto & de Morais, 2020; Santos et al., 2018), *Metschnikowia* (Lachance et al., 2001; Pozo et al., 2016), *Moniliella* (Rosa et al., 2009; Thanh & Hien, 2019) and *Candida* (Pimentel et al., 2005; Rosa et al., 2007). Species of the former two genera produce antimicrobial compounds that may aid the bee host in protection against pathogens (de Clercq et al., 2021; Lemos Junior et al., 2020; Sipiczki, 2020). *Metschnikowia* was reported to attract pollinators by producing volatile metabolites (Rering et al., 2018; Yang et al., 2019). *Moniliella* was the only basidiomycetous yeast among the dominant

ASVs in the present study. Some *Moniliella* strains produce pectinase which might provide the host with access to nutrients (Singh & Kumar, 2018). The role of *Candida* species within this niche remains unknown, but as nectivorous yeasts can alter nectar and pollen chemistry (de Vega et al., 2009; Paludo et al., 2018), they may play a role in host nutrition or pollinator attraction.

Bacterial core ASVs are shared with co-foraging bees

Although the bacterial and fungal communities of *M. sculpturalis* in Marseille were highly distinctive from those of the native bees *A. florentinum* and *H. scabiosae* (Figure 2), five out of eight core bacterial ASVs of *M. sculpturalis*, that is, *B. borstelensis*, *Brevibacillus*, *A. facilis*, *Bacillus* and *Lactococcus*, were shared with the core ASVs of *A. florentinum* and *H. scabiosae* (Figure 4a), highlighting the possibility that transmission of microbes may occur between co-foraging bees in invaded regions. Both *A. florentinum* and *H. scabiosae* have an overlapping flight period with *M. sculpturalis* and are likely to forage on the same resources. *Lythrum salicaria*, *Lavandula* sp. and *Buddleja davidii* were reported as host plants of both *A. florentinum* and *M. sculpturalis* in Southern Europe (Le Féon et al., 2021; Fortunato et al., 2013). Flowers visited by the same insects function as hubs for microbial transmission (Keller et al., 2021) and solitary bees often share microbes with visited flowers (McFrederick et al., 2017; McFrederick et al., 2018). Four core ASVs (i.e., *B. borstelensis*, *Brevibacillus*, *A. facilis* and *Bacillus*) were shared between *M. sculpturalis* and *A. florentinum*. These two species are both members of the family Megachilidae and share similar life history traits such as cavity nesting which may facilitate microbe sharing. The bacterial and fungal communities of *H. scabiosae* and the bacterial community of *A. florentinum* differed between the two sampling locations in Marseille, and a higher number of differentially abundant ASVs were detected in Parc Bonneveine/Bortoli (Figure S6, location 4B/C; Tables S3, S4). Although the two sampling locations were only 1.5 km apart, different landscape variables and ecosystems at each site may lead to the presence of niche-specific microbes. Remarkably, male and female *A. florentinum* bees differed significantly in their bacterial, but not fungal, gut community. Similarly, differences between honey bee workers and drones have been reported, and linked caste and behaviour to gut microbial composition (Kapheim et al., 2015; Yun et al., 2018). The outstanding aggressive behaviour reported for male *A. florentinum* bees (Wirtz et al., 1992), could to an extent also contribute to the observed difference in bacterial community.

Parasite absence in *M. sculpturalis*

Our study revealed an absence of microparasites in *M. sculpturalis*, both in native and invaded regions. This may suggest resistance to pathogens in the new geographical range, and, if so, likely contributes to its invasion success. Similarly, Japanese honey bees did not show compatibility with parasites of the European honey bee, also suggesting a geographic host barrier (Morimoto et al., 2013). Exotic populations of invasive species often show a reduced parasite prevalence compared to native populations (Torchin et al., 2003) and local adaptation of pathogens might prevent infection of the newly arrived bee species (Ebert, 1994). While no parasites were found in *M. sculpturalis*, the co-foraging megachilid species *A. florentinum* (native to Southern Europe) showed a parasite prevalence of 25% in Marseille. In contrast, only one out of 22 *H. scabiosae* bees was parasite-infected, suggesting a low parasite prevalence in this eusocial wild bee. Immunity in solitary bees, as well as in eusocial wild bees like *Halictus* species is barely studied. Gregarines present in *A. florentinum* had sequences highly similar to those of *Ap. bombi* and *Ap. cryptica* (Table S7). While *Ap. bombi* has also been detected in native regions of *M. sculpturalis* (Morimoto et al., 2013), two mechanisms could prevent infection: different haplotypes could occur in Marseille (Maharramov et al., 2013), or the introduced subpopulation of *M. sculpturalis* could be less susceptible to parasites. The latter could then contribute to its invasion success. Microsporidia and Trypanosoma parasites detected (mainly) in *A. florentinum* had sequences that were most similar to sequences of pathogens in non-bee hosts but that were closely related to parasite genera that commonly occur in bees as well (Table S7, data not shown). Solitary bees might host related but different parasite species compared to the extensively studied honey bees and bumble bees, as suggested by the greater phylogenetic distance towards known parasite species of social bees, like *Crithidia mellificae* in honey bees, or *Nosema bombi* in bumble bees (Figures S4, S5).

Gut microbiota shift or founder effect?

A most remarkable finding of the present study was that the gut microbial communities of *M. sculpturalis* in both invaded regions, that is, the USA and France, were highly similar and distinct from the rather diverse communities observed in a limited number of Japanese *M. sculpturalis* bees (Figure 1). Different environmental conditions are often encountered in introduced regions, including novel floral and nesting resources, biotic networks, and climate and landscape variables. This environmental context is an important factor that shapes the gut microbiome composition in other solitary bees (Cohen et al., 2020; Kapheim et al., 2021). While it is plausible to speculate that the gut microbiome of *M. sculpturalis* has shifted after its introduction into invaded regions because of changes in environmental variables or exchange of microbes with native species, this does not explain why *M. sculpturalis* gut microbiota compositions in both invaded regions are so similar. New York and Marseille were recently identified as highly suitable areas for *M. sculpturalis* (Lanner et al., 2022). However, comparison of bioclimatic and landscape variables for the sampling sites retrieved from CHELSA version 2.1 (Karger et al., 2017) and Land Use Harmonisation dataset version 2 (Chen et al., 2020), respectively, showed distinct temperature, precipitation and land use conditions for both locations (data not shown). Remarkably, at both locations *M. sculpturalis* was repeatedly

documented to forage preferentially on pollen from exotic species such as the ornamental Japanese pagoda tree (*Styphnolobium japonicum*) (Le Féon et al., 2018; Matteson et al., 2008; Parys et al., 2015; Quaranta et al., 2014). In the present study, 58% of the ITS2 reads detected in *M. sculpturalis* from both invaded regions were attributed to *S. japonicum*, whereas *S. japonicum* reads were nearly absent in the *M. sculpturalis* bees collected in Japan (data not shown). This suggests that foraging preference towards *S. japonicum* pollen in invaded regions may contribute to the development of a uniform gut microbiome.

Alternatively, a founder effect may also explain observations of the present study. Founder effects have been described earlier to explain reduced genetic variation in isolated populations (Alsos et al., 2015; Geng et al., 2021; Hedrick et al., 2001) and the absence of parasites (Torchin et al., 2003). Although a limited number of samples could be collected from its native region in Japan, the gut microbial composition of *M. sculpturalis* bees in Japan was highly diverse (Figure 1a). We hypothesize that native *M. sculpturalis* populations display diverse gut microbiome communities and include bees with a gut microbiome community as observed in the invaded regions. The examination of additional samples from native regions may therefore reveal a gut microbiome community as observed in France and the USA. The latter native *M. sculpturalis* bees may be more prone to invasion and pathogen free, and population reestablishment in the invaded regions may have led to the occurrence of a more homogeneous microbiota. This would imply that the gut microbial community of such invasive bees is hardly modified by entry in the new environment, or that an unknown factor in the invasive bees selects for the same environmental bacteria. While comprehensive functional microbiota studies can primarily be performed with axenic isolates and functional genome analyses, the present identification results revealed dominant gut bacteria and fungi that are primarily known as environmental microorganisms (Figure 1). While these gut microbes likely contribute some competitive advantages to their host (Maebe et al., 2021), this does not explain the rather exceptional invasive success of *M. sculpturalis*.

EXPERIMENTAL PROCEDURES

Sample collection

Foraging *M. sculpturalis* were collected from Japan (native region), the USA (invaded) and France (invaded) in the summer of 2020 (Figure S6). Samples from Japan and the USA were collected in August and September by collaborators at the locations. The sampling locations in Japan included Mukuchi Island, Okayama ($n = 2$) and Tsukuba City, Ibaraki ($n = 4$). Samples from the USA were all collected in New York City ($n = 4$). In France, *M. sculpturalis* bees were collected in Font-Romeu ($n = 9$) in August and at different urban parks in Marseille ($n = 27$) in July. A sampling of the co-foraging bees *A. florentinum* ($n = 28$) and *H. scabiosae* ($n = 22$) was performed simultaneously at the same sites in Marseille (Figure S6). Ten sampling sites were visited in Marseille, but only six yielded sufficient samples and were included in the present study. Sampling at each site or location was performed only once, except in New York, where multiple visits were required. All sampling locations and bee species are listed in Table S1. All specimens were stored at −20°C until use. Native bee species (*H. scabiosae* and *A. florentinum*) were identified using the field guide for bees of Europe (Michez et al., 2019), and identification of *M. sculpturalis* was done with the exotic bee ID tool (Burrows et al., 2021).

Bee processing and DNA extraction

All bees were dissected under sterile conditions after surface-sterilisation using Ummonium38 Medical Spray. The whole gut was extracted and homogenised in 275 µL of STET buffer (8% sucrose, 5% Triton X-100, 50 mM EDTA and 50 mM Tris–HCl) using sterile micropestles. Total bacterial, fungal and parasite DNA was extracted as described by Snauwaert et al. (2016) with following minor modifications. The STET supernatant was collected in a separate 1.5 mL Eppendorf tube after initial centrifugation and was used to resuspend the pellet before the lysis step. DNA quality was assessed via NanoDrop and electrophoresis on a 1% agarose gel. DNA concentration was measured using a Quantus™ Fluorometer.

Amplicon sequencing of the bacterial and fungal community

A subset of 49 DNA samples (25 *M. sculpturalis*, 15 *A. florentinum* and 9 *H. scabiosae*) (Table S1) were sent to BaseClear B.V. (Leiden, The Netherlands) for library preparation and amplicon sequencing. The 16S rRNA V3-V4 region was PCR amplified using the primer pair 341F (5'-CCTACGGGNGGCWGCAG-3′) and 785R (5'-GACTACHVGGGTATCTAATCC-3′). The fungal internal transcribed spacer 2 region (ITS2) was PCR amplified using the primer pair ITS3F (5'-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5'-TCCTCCGCTTATTGATATGC-3′). The library was sequenced on an Illumina MiSeq platform generating 300 bp paired-end reads.

16S rRNA and ITS2 raw sequencing reads were analysed separately with the DADA2 pipeline version 1.16 (Callahan et al., 2016). After read quality inspection, the bacterial forward and reverse reads were trimmed to a length of 280 bp and 230 bp, respectively, and primers were removed from the respective reads using the trimLeft parameter. Merged paired reads with a length between 401 bp and 428 bp were retained for further analyses. ITS2 primers were removed using cutadapt version 3.4 (Martin, 2011) and reads with a minimum length of 50 bp were retained through the rest of the pipeline. Taxonomy was assigned to the resulting 16S rRNA and ITS2 amplicon sequence variants (ASVs) using the DADA2 formatted training FASTA files of the SILVA SSU database version 138.1 (McLaren & Callahan, 2021) and the General FASTA release files from the UNITE ITS database version 8.3 (All eukaryotes) (Abarenkov et al., 2021).

ASVs attributed to chloroplasts, mitochondria, Archaea, Eukarya and unclassified phyla were removed from the 16S rRNA dataset, whereas only ASVs assigned to fungi were retained in the ITS2 dataset for further analysis.

Microbial community analysis

All analyses were performed in R version 4.1.0. Alpha diversity (Observed richness and Shannon diversity) and beta diversity measures were calculated and plotted using the phyloseq package version 1.36.0 (McMurdie & Holmes, 2013).

Comparison of alpha diversity estimates between sampling locations of *M. sculpturalis* and between bee species in Marseille was performed with the Kruskal-Wallis test and pairwise comparisons with the Wilcoxon rank-sum test. Statistical tests were applied using the ggpubr package version 0.4.0. Beta diversity was assessed through calculation of Bray–Curtis dissimilarity matrices and visualised through PCoA plots. Community dissimilarity between the sampling locations of *M. sculpturalis*, between bee species in Marseille, between bee sex and between sampling sites in Marseille were evaluated using analysis of homogeneity of group dispersions ('betadisper') and PERMANOVA statistical test ('adonis' with 9999 permutations) available in the vegan package version 2.5-7 (Oksanen et al., 2020). Pairwise PERMANOVA tests were performed using the pairwiseAdonis package version 0.4. Differentially abundant taxa ($p_{adj} < 0.05$) between groups were identified using the DESeq2 package version 1.32.0 (Love et al., 2014) considering only ASVs constituting at least 1% of the relative abundance. The *p*-values were adjusted for multiple hypothesis testing using the Benjamini–Hochberg method for all pairwise statistical tests. Bacterial and fungal core taxa were defined as taxa being present in at least 50% of the samples at a relative read abundance greater than 1% (Graystock et al., 2017).

Parasite detection

Primers targeting an 18S rRNA gene fragment were designed for PCR-based detection of Microsporidia, Metakinetoplastea and Gregarinorida (Table S6; sets 1A, 2A, 3A), and were ordered via a commercial company (Integrated DNA Technologies, Leuven, Belgium). PCR products were visualised via electrophoresis on a 1.5% agarose gel to confirm the expected amplicon size (Table S6). Samples that yielded PCR amplicons of the expected size were further analysed using four sets of genus-specific PCR primers (Table S6; sets 1B, 2B, 3B, 3C) and the obtained amplicons were Sanger sequenced using the same primers (LGC Genomics, Berlin, Germany). The obtained sequences were analysed via NCBI BLAST (Johnson et al., 2008) for tentative species-level identification of parasites. A distance tree was created based on pairwise alignments in NCBI BLAST and included nearest phylogenetic neighbour species and sequences of established bee parasite species of the same families. The treeing method used was Fast Minimum Evolution with a Max Seq difference of 0.75.

CONCLUSION

We provided a comprehensive analysis of the gut microbial communities associated with the invasive bee *M. sculpturalis* in its native (Japan) and invaded (USA and France) geographical range. Its microbiome composition differed significantly between native and invasive bees, and differed from that of native co-foraging bees in Marseille. Core ASVs were assigned to environmental microbes that likely provide beneficial functions to their host. Some core ASVs were shared with French native bees, suggesting direct or indirect transmission. *Megachile sculpturalis* bees examined in the present study did not harbour known bee microparasites. Two hypotheses may explain the microbial community profiles and the absence of parasites: a common shift in gut microbiota in the invaded regions as a response to changed environmental conditions, or a founder effect in gut microbial composition caused by the introduction events.

AUTHOR CONTRIBUTIONS

Tina Tuerlings: Conceptualization; investigation; writing – original draft; writing – review and editing; methodology; resources; data curation. **Amanda Hettiarachchi:** Conceptualization; investigation; writing – original draft; writing – review and editing; formal analysis; visualization; data curation; methodology. **Marie Joossens:** Writing – review and editing; formal analysis. **Benoît Geslin:** Writing – review and editing; methodology; resources. **Nicolas J. Vereecken:** Funding acquisition; writing – review and editing. **Denis Michez:** Writing – review and editing; funding acquisition. **Guy Smagghe:** Conceptualization; writing – original draft; funding acquisition; writing – review and editing. **Peter Vandamme:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

16S rRNA and ITS2 amplicon sequencing data generated for the present study are archived at the European Nucleotide Archive and are accessible through BioProject numbers PRJEB53414 and PRJEB53998, respectively.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Number of bee specimens sampled from different locations for the present study. The subset of samples used for 16S rRNA and ITS2 amplicon sequencing are given in brackets.

Table S2. Pairwise PERMANOVA tests for each group pairing to test for significant pairwise differences in Bray–Curtis dissimilarity matrices of the bacterial and fungal communities. The *p* values were adjusted using the Benjamini–Hochberg method. Significant values ($p_{adj} < 0.05$) are indicated in bold. AF, *Anthidium florentinum*; HS, *Halictus scabiosae*; MS, *Megachile sculpturalis*.

Table S3. Significantly differentially abundant bacterial ASVs ($p_{adj} < 0.05$) between the analysed group pairings, as detected through DESeq2 analysis. We used only ASVs with a minimum relative abundance of 1% in the samples.

Table S4. Significantly differentially abundant fungal ASVs ($p_{adj} < 0.05$) between the analysed group pairings, as detected through DESeq2 analysis. We used only ASVs with a minimum relative abundance of 1% in the samples.

Table S5. Pairwise Wilcoxon tests to test pairwise significant differences in alpha diversity estimates (Observed richness and Shannon diversity) between analysed group pairings. The *p* values were adjusted using the Benjamini–Hochberg method. Significant values ($p_{adj} < 0.05$) are indicated in bold. AF, *Anthidium florentinum*; HS, *Halictus scabiosae*; MS, *Megachile sculpturalis*.

Table S6. Primers used in the present study for the detection of parasites. All primer sets target a fragment of the 18S rRNA gene, except for set 2B which targets the internal transcribed spacer 1 region.

Table S7. Comparative sequence analysis-based closest neighbours of parasite species detected in the present study. Sequence analyses were all performed with partial 18S rRNA sequences.

Figure S1. Rarefaction curves of each sample at various sequencing depths for (A) 16S rRNA and (B) ITS2 sequences. AF, *Anthidium florentinum*; HS, *Halictus scabiosae*; MS, *Megachile sculpturalis*.

Figure S2. Alpha diversity estimates of *Megachile sculpturalis* for the different sampling locations: observed richness of the (A) bacterial and (C) fungal communities and Shannon diversity of the (B) bacterial and (D) fungal communities. Kruskal-Wallis tests indicate alpha diversity was significantly different between groups ($p < 0.05$). Pairwise Wilcoxon tests were performed of every group pairing to test for pairwise differences (Table S5).

Figure S3. Alpha diversity estimates of the different bee species sampled in Marseille: observed richness of the (A) bacterial and (C) fungal communities and Shannon diversity of the (B) bacterial and (D) fungal communities. Kruskal–Wallis tests indicate alpha diversity was significantly different between groups ($p < 0.01$). Pairwise Wilcoxon tests were performed of every group pairing to test for pairwise differences (Table S5).

Figure S4. Phylogenetic distance tree of the partial 18S rRNA gene amplicon, earlier classified as *Crithidia* sp. using genus-specific primers, from a specimen of *Anthidium florentinum*, generated by primer set 2A of Table S6. Tree constructed via the BLAST tool of NCBI with default method of Fast Minimum Evolution. Scale shows evolutionary distance of 0.01. *Crithidia mellificae* and *Crithidia bombi* sequences were added to compare with the 5 sequences showing the same sequence similarity to the partial 18S rRNA gene amplicon.

Figure S5. Phylogenetic distance tree of the partial 18S rRNA gene amplicon, earlier classified as *Nosema* sp. using genus-specific primers, from a specimen of *Anthidium florentinum*, generated by primer set 1A of Table S6. Tree constructed via the BLAST tool of NCBI with default method of Fast Minimum Evolution. Scale shows evolutionary distance of 0.01. *Nosema thomsoni* and *Nosema bombi* sequences were added to compare with the two sequences showing the same sequence similarity to the partial 18S rRNA gene amplicon.

Figure S6. Sampling locations of *Megachile sculpturalis* and co-foraging bees. Mukuchi Island, Okayama (1) and Tsukuba City, Ibaraki (2) in Japan represent the native range of *M. sculpturalis*. New York City, USA (3), Marseille, France (4) and Font-Romeu, France (5) represent the invaded regions. An elaborate sampling was performed in different parks in Marseille to collect native bees co-foraging with *M. sculpturalis*: Parc Borély (4A), Parc Bonneveine (4B), Parc Bortoli (4C), Parc de l'Oasis (4D), Parc Athéna (4E) and Parc de la Buzine (4F). Maps were created with Datawrapper.

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