



# Low prevalence of secondary endosymbionts in aphids sampled from rapeseed crops in Germany

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## Research Paper

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### Abstract

Peach-potato aphids, *Myzus persicae* Sulzer (Hemiptera:Aphididae), and cabbage aphids, *Brevicoryne brassicae* Linnaeus (Hemiptera:Aphididae), are herbivorous insects of significant agricultural importance. Aphids can harbour a range of non-essential (facultative) endosymbiotic bacteria that confer multiple costs and benefits to the host aphid. A key endosymbiont-derived phenotype is protection against parasitoid wasps, and this protective phenotype has been associated with several defensive endosymbionts. In recent years greater emphasis has been placed on developing alternative pest management strategies, including the increased use of natural enemies such as parasitoid wasps. For the success of aphid control strategies to be estimated the presence of defensive endosymbionts that can potentially disrupt the success of biocontrol agents needs to be determined in natural aphid populations. Here, we sampled aphids and mummies (parasitised aphids) from an important rapeseed production region in Germany and used multiplex PCR assays to characterise the endosymbiont communities. We found that aphids rarely harboured facultative endosymbionts, with 3.6% of *M. persicae* and 0% of *B. brassicae* populations forming facultative endosymbiont associations. This is comparable with endosymbiont prevalence described for *M. persicae* populations surveyed in Australia, Europe, Chile, and USA where endosymbiont infection frequencies range from 0–2%, but is in contrast with observations from China where *M. persicae* populations have more abundant and diverse endosymbiotic communities (endosymbionts present in over 50% of aphid populations).

## Introduction

Aphids, such as the peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera:Aphididae), and the cabbage aphid, *Brevicoryne brassicae* Linnaeus (Hemiptera:Aphididae), are important insect herbivores (Edde, 2021). Aphids are pests on many crops, including rapeseed (Zheng *et al.*, 2020), where crop damage is caused through direct feeding (Dedryver *et al.*, 2010) and the transmission of Turnip Yellows Virus, TuYV, (Asare-Bediako *et al.*, 2020). Aphids reduce rapeseed yields directly through feeding activities and indirectly via the transmission of plant viruses: Depending on the rapeseed variety and crop growth stage at the time of inoculation, TuYV infection can reduce yields by 26–40% (Congdon *et al.*, 2020).

Currently, aphid populations are primarily controlled using insecticides. However, due to more stringent regulations (EU 2009), greater environmental concerns around insecticide use (Goulson, 2013), and the emergence of aphid populations with insecticide resistance or reduced insecticide sensitivity (Bass *et al.*, 2015; Bass and Nauen, 2023), there is a growing need for more sustainable pest management practices (Ali *et al.*, 2023). One avenue that is being explored is the promotion of natural enemy populations, such as parasitoid wasps, that can provide natural pest regulation services (Ali *et al.*, 2023; Elliott *et al.*, 2023). The main parasitoids of *M. persicae* and *B. brassicae* include *Aphidius ervi* Haliday (Hymenoptera: Braconidae), *A. colemani* Viereck (Hymenoptera: Braconidae), and *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae) (Mehrpour *et al.*, 2019; Ward *et al.*, 2022). By promoting the abundance and activities of these parasitoids farmers could increase the provision of natural pest regulation services and reduce reliance on chemical-based pest control methods. However, aphid-encoded factors can influence the success of these parasitoids.

The majority of aphid species form an essential (obligate) relationship with the endosymbiont *Buchnera aphidicola*. *B. aphidicola* supplements the aphid diet by providing access to essential amino acids (Douglas and Prosser, 1992). Aphids can also form a diverse range of non-essential (facultative) endosymbiotic relationships. These facultative endosymbionts are key drivers of phenotypic diversity in aphids (Zytynska *et al.*, 2021) and around nine facultative endosymbionts have been described (Zytynska and Weisser, 2016). The most common

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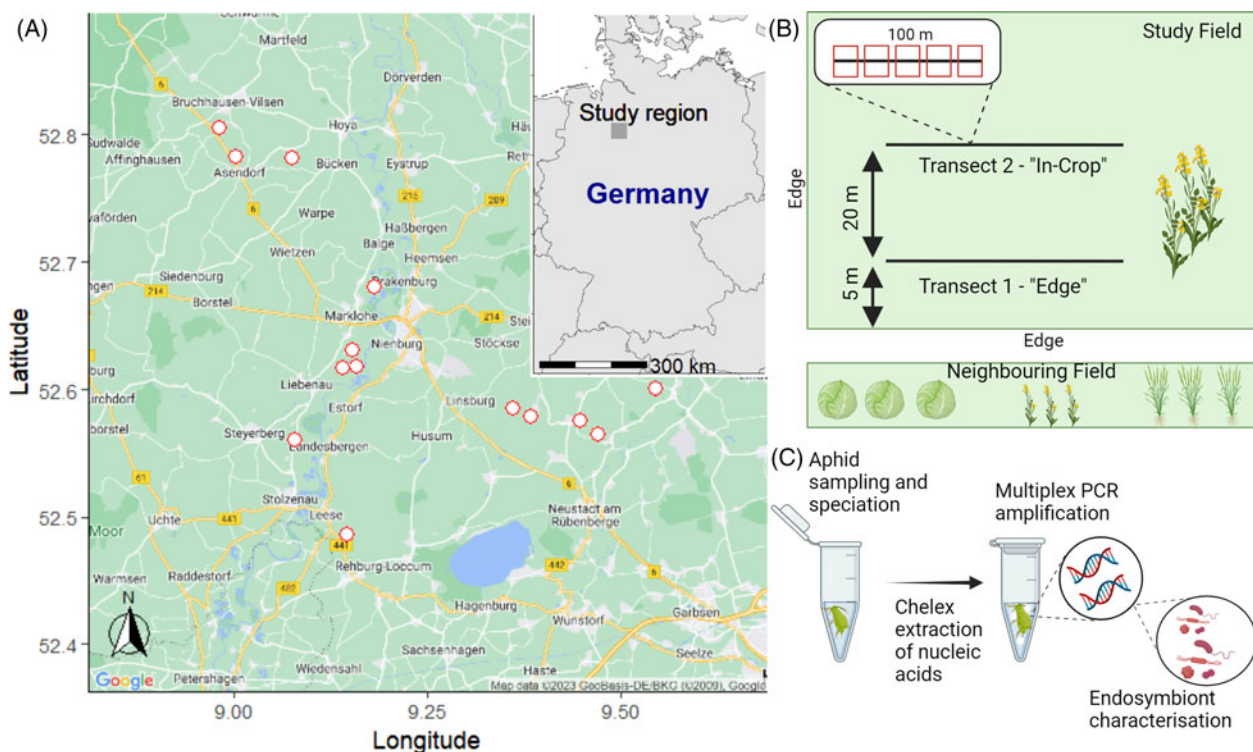


facultative endosymbionts that have been described to associate with aphids are *Spiroplasma* spp., *Regiella insecticola*, *Hamiltonella defensa*, *Rickettsiella* sp., *Fukatsuiia symbiotica* (previously pea aphid x-type symbiont, PAXS; Patel *et al.*, 2019), *Serratia symbiotica*, *Rickettsia* spp., *Arsenophonus* spp., and *Wolbachia* spp. (Zytnyska and Weisser, 2016). Associations with endosymbionts can occur in single infections (i.e., one facultative endosymbiont), co-infections (two endosymbionts), or multiple co-occurring infections (Zytnyska *et al.*, 2023).

Facultative endosymbionts influence the phenotype of many aphid species (Zytnyska and Weisser, 2016; Zytnyska *et al.*, 2021) and a key endosymbiont-derived phenotype is protection against parasitoid wasps (Oliver and Higashi, 2019; Zytnyska *et al.*, 2021). In most aphid species protection against parasitism is conferred by the defensive endosymbiont, *H. defensa* (Leybourne *et al.*, 2020; Zytnyska *et al.*, 2021). Other endosymbionts can also provide protective services, for example in *M. persicae* protection against parasitism is associated with *R. insecticola* (von Burg *et al.*, 2008; Vorburger *et al.*, 2009). It has also been suggested that facultative endosymbionts, including these defensive endosymbionts, can influence aphid susceptibility to insecticides (Li *et al.*, 2021). However, these observations were made by manipulating aphid endosymbiont communities in controlled environments (Li *et al.*, 2021) and were not observed for aphid populations with naturally occurring endosymbiont associations (Leybourne *et al.*, 2023). For the potential success of aphid control strategies to be better estimated the natural prevalence of potentially defensive endosymbionts in aphid populations needs to be determined, particularly for endosymbionts that can potentially disrupt the success of biocontrol agents such as parasitoid wasps.

The endosymbionts of *M. persicae* have been surveyed for several geographic regions, including Australia (Yang *et al.*, 2023a; Yang *et al.*, 2023b), China (Xu *et al.*, 2021; Yang *et al.*, 2023b), Colombia (Gallo-Franco *et al.*, 2019), and The Netherlands (Beekman *et al.*, 2022). Endosymbionts in these *M. persicae* populations include *R. insecticola* (potentially defensive), *Wolbachia* spp., *Rickettsia* spp., *Arsenophonus* spp., *S. symbiotica*, *H. defensa* (potentially defensive), and *Spiroplasma* spp. (von Burg *et al.*, 2008; Vorburger *et al.*, 2009; Henry *et al.*, 2015; Xu *et al.*, 2021; Yang *et al.*, 2023b). The prevalence of endosymbionts in *M. persicae* is highly variable, with some populations forming no endosymbiotic associations (Yang *et al.*, 2023a) and others showing high levels (>40%) of endosymbiont infection (Xu *et al.*, 2021). For *B. brassicae*, several studies have screened for endosymbionts but no associations have been detected for this aphid (Clark *et al.*, 2012; Yang *et al.*, 2023a).

Here, we sampled *M. persicae* and *B. brassicae* populations, alongside mummified aphids, from 14 winter rapeseed fields. We sampled aphids along two transects, one next to the field edge and the second within the crop, and used a multiplex diagnostic PCR method to characterise the facultative endosymbiont community for each aphid population. Aphids were sampled from a key rapeseed production region in Lower Saxony, Germany (fig. 1A). Sampling occurred between 26–28th October 2021, a time-period that reflects peak autumn aphid activity and virus risk (Ellis *et al.*, 2014). The aim of our research was to gain insights into the prevalence of potentially defensive endosymbionts in naturally-occurring aphid populations for these two agriculturally important aphid species.



**Figure 1.** (A) Study region (shaded grey box) in Lower Saxony, North Germany and location of the 14 study sites (white circles). (B) Graphical representation of the trial design followed at each field site: Each field site was adjacent to another agricultural field with the first transect 5 m away from the field edge and the second transect 20 m into the crop; each transect contained five 2 m<sup>2</sup> quadrats. (C) Overview of the DNA extraction process. Maps were created in ggmap (v.3.0.2) with the base map obtained from Google Map Services. This graphic was prepared in bio-render.

## Methods

### Sampling procedure

We sampled 14 winter rapeseed fields in an agriculturally important region of Lower Saxony, Germany (fig. 1A). In each field, sampling occurred along two 100 m transects (fig. 1B). One was 5 m from the field edge and the second 20 m into the crop. We sampled at two distances into the field as recent results in cereal aphids indicate that this can influence the prevalence and diversity of aphid endosymbionts (Zytynska *et al.*, 2023); as agricultural systems are not homogenous we did not want to exclusively sample from the centre of the crop as this would not provide a realistic representation of the aphid and endosymbiont communities. Transects were divided into five equidistant sampling regions and a 2 m<sup>2</sup> quadrat was surveyed at each sampling region. One aphid colony (hereafter population) was sampled per quadrat. The number of individual aphids per population ranged from one to 20 and all aphids were pooled into one sample. Where present, an aphid mummy was also collected from each quadrat. Mummies were identified morphologically. Aphid and mummy samples were stored at -20 °C until DNA extraction.

### DNA extraction and PCR

DNA was extracted using the Chelex® method: A 5% Chelex® 100-resin (Bio-Rad, Germany) solution was made using UltraPure water (Invitrogen, Germany) and heated to c. 60 °C. 10 µl Chelex® solution and 1.5 µl Proteinase K (20 mg L<sup>-1</sup>, Macherey-Nagel GmbH & Co. KG, Germany) were added to each sample. Aphids were homogenised using sterile pipette tips and mummies with a sterile micropestle. After homogenisation, 80 µl Chelex® solution was added, the sample was vortexed and incubated for 1.5 h at 56 °C. After incubation, samples were centrifuged at max speed for 5 minutes and the solution was transferred to a clean Eppendorf tube, diluted 1:2 with TE buffer (10 mM Tris-HCl pH 7.5; 1 mM EDTA pH 8.0), and stored at -20 °C. An extraction blank was included with each batch of DNA extractions.

Successful DNA extraction was confirmed using a PCR marker for the obligate endosymbiont *B. aphidicola*. The presence of facultative endosymbionts was determined using multiplex assays (Beekman *et al.*, 2022). Assays were conducted in a Biometra TRIO 48 Thermocycler (Analytik Jena, Germany). Primer and thermocycling details are described in Table S1. Successful amplification was detected by product separation on a 1% agarose gel stained with GelRed® (Biotium, Germany).

## Results and discussion

From the 140 sampling regions (14 fields × 10 quadrats) we sampled 114 aphid populations and 20 mummies, with DNA successfully extracted from 119 of these samples (indicated by detection of *B. aphidicola*; Table 1). Aphid samples primarily comprised *M. persicae* (Table 1). Our results indicate that *M. persicae* and *B. brassicae* rarely harboured facultative endosymbionts. From the 116 *M. persicae* populations (109 successful aphid extractions + seven successful mummy extractions; Table 1) we detected facultative endosymbionts in four populations – a 3.6% infection frequency. Endosymbionts included one single *Rickettsiella* spp. infection, two single *H. defensa* infections, and one co-infection (*S. symbiotica* and *Rickettsia* spp.). Previous studies characterising endosymbionts in *M. persicae* populations

found similarly low levels of endosymbiont prevalence. In the Netherlands no facultative endosymbionts were detected in 780 *M. persicae* samples collected from peppers (Beekman *et al.*, 2022), a global study found <2% facultative endosymbiont infection frequencies (screening for *H. defensa*, *R. insecticola*, and *S. symbiotica*) in 50 *M. persicae* populations sampled from a range of crop plants (Henry *et al.*, 2015), a survey of *M. persicae* from pepper crops in Colombia found no facultative endosymbiont associations (Gallo-Franco *et al.*, 2019), and a recent global survey found no endosymbiont associations in 52 *M. persicae* populations sampled across Australia, Europe, USA, and Chile (Yang *et al.*, 2023b). Alongside our current observations, these findings indicate that *M. persicae* rarely form facultative endosymbiotic associations (Henry *et al.*, 2015; Gallo-Franco *et al.*, 2019; Beekman *et al.*, 2022).

Conversely, facultative endosymbionts have been detected at much higher frequencies in *M. persicae* sampled from China (Xu *et al.*, 2021; Yang *et al.*, 2023b). A greater frequency and diversity of endosymbionts were reported by Xu *et al.* (2021) where the main endosymbionts present in aphid populations were *Wolbachia* spp. (present in 57% of samples), *Rickettsia* spp., *Arsenophonus* spp., and *S. symbiotica* (16% of samples), *R. insecticola* (13% of samples), *H. defensa* (4% of samples), and *Spiroplasma* spp. (3% of samples). In Yang *et al.* (2023b) only two endosymbionts were detected, namely *Rickettsia* spp. (present in 19% of samples), and *Spiroplasma* spp. (4% of samples), but these were at a higher prevalence than observed in aphids surveyed from other regions. The endosymbiont frequencies described in Xu *et al.* (2021), Yang *et al.* (2023b) are greater than those detected in *M. persicae* sampled from other geographical areas, including Europe, Australia, USA, and Chile (Henry *et al.*, 2015; Gallo-Franco *et al.*, 2019; Beekman *et al.*, 2022). This indicates that the endosymbiont communities in *M. persicae* might differ depending on bio-geographic location, as previously suggested for cereal aphids (Guo *et al.*, 2019).

There are several potential explanations for this consistent observation of greater diversity and prevalence of endosymbionts in *M. persicae* sampled from China when compared with aphid populations from other regions (this study; Henry *et al.*, 2015; Xu *et al.*, 2021; Beekman *et al.*, 2022; Yang *et al.*, 2023b). Variation in endosymbiont communities between native vs invasive populations is one explanation that has recently been suggested (Yang *et al.*, 2023b). Yang *et al.*, 2023b observed that *M. persicae* sampled in their putative native habitat (China) had a greater diversity, with regards to both genetic and microbial diversity, than invasive *M. persicae* populations sampled from Europe, Australia, or America. This is in-line with observations in similar aphid species where native populations can have a greater microbial diversity when compared with invasive (exotic) populations (Desneux *et al.*, 2018). Although this phenomena might be species-specific as little difference was detected between natural and invasive soybean aphid, *Aphis glycines* Matsumura, populations (Bansal *et al.*, 2014).

Host plant diversity is another potential explanation. Recent controlled environment studies have shown that host plant diversity can influence and modulate the microbiome of *M. persicae* (He *et al.*, 2021); host plant diversity could also influence the microbiome of *M. persicae* in natural aphid populations. Most surveys have focussed on sampling *M. persicae* from agricultural systems, where host plant diversity is relatively low (this study; Henry *et al.*, 2015; Beekman *et al.*, 2022; Yang *et al.*, 2023b). However, Xu *et al.*, 2021 collected *M. persicae* from a diverse



**Table 1.** Details on the total number of samples (aphid or mummy) collected for each field alongside endosymbiont prevalence

Field	Sample type	Number of populations sampled	Endosymbiont infection frequency										
			<i>B.a</i>	<i>Spi</i>	<i>R.i.</i>	<i>H.d.</i>	<i>R-siella</i>	<i>F. s.</i>	<i>S.s.</i>	<i>R-tsia</i>	<i>Ars.</i>		
2	<i>M. persicae</i>	6	6/6	No facultative endosymbionts detected									
3	<i>M. persicae</i>	2	2/2	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	1	1/1	No facultative endosymbionts detected									
5	<i>M. persicae</i>	6	6/6	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	3	1/3	No facultative endosymbionts detected									
6	<i>M. persicae</i>	9	9/9	No facultative endosymbionts detected									
16	<i>M. persicae</i>	6	6/6								1/6		
	<i>B. brassicae</i>	1	1/1	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	2	0/2	No facultative endosymbionts detected									
17	<i>M. persicae</i>	8	8/8	1/8				1/8: co-infection					
	Mummy ( <i>M. persicae</i> )	3	1/3	No facultative endosymbionts detected									
18	<i>M. persicae</i>	9	8/9	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	4	1/4								1/1		
20	<i>M. persicae</i>	7	7/7	No facultative endosymbionts detected									
	<i>B. brassicae</i>	2	2/2	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	4	1/4	No facultative endosymbionts detected									
21	<i>M. persicae</i>	11	11/11	No facultative endosymbionts detected									
25	<i>M. persicae</i>	9	9/9	No facultative endosymbionts detected									
27	<i>M. persicae</i>	11	11/11	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	1	1/1	No facultative endosymbionts detected									
30	<i>M. persicae</i>	10	10/10	No facultative endosymbionts detected									
31	<i>M. persicae</i>	9	8/9	No facultative endosymbionts detected									
	Mummy ( <i>M. persicae</i> )	2	1/2	No facultative endosymbionts detected									
32	<i>M. persicae</i>	8	8/8	No facultative endosymbionts detected									

Symbiont abbreviations: *B.a* (*B. aphidicola*; essential primary endosymbiont – used to confirm DNA extraction), *Spi* (*Spiroplasma* spp.), *R.i.* (*Regiella insecticola*), *H.d.* (*Hamiltonella defensa*), *R-siella* (*Rickettsiella* sp.), *F.s.* (*Fukatsuiella symbiotica*), *S.s.* (*Serratia symbiotica*), *R-tsia* (*Rickettsia* spp.), and *Ars.* (*Arsenophonus* spp.).

range of host plants, not solely from agricultural fields. This could potentially explain the high diversity reported for *M. persicae* in Xu *et al.*, 2021, particularly when compared with a slightly lower endosymbiont prevalence in *M. persicae* sampled from agricultural fields in China (Yang *et al.*, 2023b). However, this requires further testing.

We detected no endosymbionts in the three *B. brassicae* populations, and a similar survey of aphid populations from Northern Britain also found no facultative endosymbiont occurrence in *B. brassicae* (Clark *et al.*, 2012). Several studies have employed 16S rDNA sequencing methods to identify uncharacterised, or cryptic, species beyond the main facultative endosymbionts. Where additional bacteria have been detected, these were primarily environmental microbes, e.g., *Klebsiella* spp., *Escherichia* spp., *Pseudomonas* spp. (Clark *et al.*, 2012; Beekman *et al.*, 2022), indicating that endosymbiotic prevalence in European *M. persicae* and *B. brassicae* populations is low.

Endosymbionts can provide strong protective traits, including resistance against parasitoid wasps (von Burg *et al.*, 2008; Vorburger *et al.*, 2009). It has been assumed that the presence

of these endosymbionts will have detrimental effects on sustainable pest management practices, with the presence of endosymbiont-infected aphids disrupting biocontrol success. Field-based research using *H. defensa*-infected and uninfected *A. fabae* in bean fields has provided experimental evidence showing that endosymbiont infection reduces parasitism rate under field conditions (Rothacher *et al.*, 2016). However, as indicated by our study and others (Henry *et al.*, 2015; Xu *et al.*, 2021; Beekman *et al.*, 2022), the prevalence of these potentially protective endosymbionts in natural aphid populations is low, indicating that the impact of endosymbionts on biocontrol success under natural conditions could be minimal (Beekman *et al.*, 2022). The role of other traits that might influence biocontrol success, such as aphid genotype, also needs to be examined in more detail.

Conversely, recent research has indicated that endosymbionts could also be used as a novel method of aphid biocontrol (Gu *et al.*, 2023; Soleimannejad *et al.*, 2023). Artificial transfection of aphids with a *Rickettsiella* symbiont (*R. viridis*) was shown to contribute towards the suppression of *M. persicae* populations (Gu *et al.*, 2023), with *R. viridis* infection reducing aphid

fecundity. *R. viridis* is thought to also be able to follow a horizontal transfer mechanism via plant-mediated transfer and can be spread throughout an aphid colony (Gu *et al.*, 2023). The interaction between this aphid-suppressing endosymbiont and the parasitoid *D. rapae* was recently assessed, with results suggesting that *D. rapae* preferred to probe aphids infected with *R. viridis* (Soleimannejad *et al.*, 2023). The authors proposed that this could potentially contribute an additional horizontal transfer mechanism for *R. viridis* (Soleimannejad *et al.*, 2023); although the successful rate of horizontal transfer of facultative endosymbionts by parasitoids is minimal (Gehrer and Vorburget, 2012). These mechanisms of *R. viridis*-mediated aphid suppression and *D. rapae*-mediated transfer of *R. viridis* could be combined into a novel biocontrol method (Soleimannejad *et al.*, 2023). However, several points need to be considered: (1) Currently these observations are made for *M. persicae* that have been artificially transfected with *R. viridis* derived from the pea aphid, *Acyrtosiphon pisum* Harris (Hemiptera:Aphididae), and the potential role of *M. persicae*-derived *Rickettsiella* spp. needs to be considered; (2) When transferred horizontally *R. viridis* is only stable for c. two generations (Soleimannejad *et al.*, 2023), therefore the potential impact this short retention might have on aphid suppression under natural conditions needs to be considered; (3) The interactions between *R. viridis* and naturally occurring endosymbionts also needs to be examined in more detail, particularly the interaction with endosymbionts that confer resistance against parasitism and the potential impact of this on *D. rapae*-mediated horizontal transfer.

## Conclusion

Our results add further evidence that facultative endosymbiont associations are rarely formed in *M. persicae* and *B. brassicae* aphids across Europe (Clark *et al.*, 2012; Henry *et al.*, 2015; Beekman *et al.*, 2022). Contrasting variation between endosymbiont frequencies in *M. persicae* from Europe (this study; Henry *et al.*, 2015; Beekman *et al.*, 2022; Yang *et al.*, 2023b) and China (Xu *et al.*, 2021; Yang *et al.*, 2023b) suggest that endosymbiont frequencies could be influenced by other environmental or bio-geographical factors; geographical variation has been detected in the endosymbionts of cereal aphids (Guo *et al.*, 2019). Recent research has suggested that native aphid populations are potentially more diverse than invasive populations (Yang *et al.*, 2023b) and highlighted the role the host plant plays in influencing endosymbiont composition (He *et al.*, 2021). Exploring these in greater detail would be interesting avenues for future research.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0007485324000063>.

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**Competing interest.** None.

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