Herbivore range expansion triggers adaptation in a subsequently-associated third trophic level species and shared microbial symbionts

Fushi Ke1,2,3, Shijun You1,2,3, Sumei Huang1,2,3, Weijun Chen1,2,3, Tiansheng Liu1,2,3, Weiyi He1,2,3, Dandan Xie1,2,3, Qiang Li1,2,3, Xijian Lin1,2,3, Liette Vasseur4,5, Geoff M. Gurr1,2,5 & Minsheng You1,2,3

Invasive species may change the life history strategies, distribution, genetic configuration and trophic interactions of native species. The diamondback moth, *Plutella xylostella* L., is an invasive herbivore attacking cultivated and wild brassica plants worldwide. Here we present phylogeographic analyses of *P. xylostella* and one of its major parasitoids, *Cotesia vestalis*, using mitochondrial markers, revealing the genetic diversity and evolutionary history of these two species. We find evidence that *C. vestalis* originated in Southwest China, then adapted to *P. xylostella* as a new host by ecological sorting as *P. xylostella* expanded its geographic range into this region. Associated with the expansion of *P. xylostella*, *Wolbachia* symbionts were introduced into local populations of the parasitoid through horizontal transfer from its newly associated host. Insights into the evolutionary history and phylogeographic system of the herbivore and its parasitoid provide an important basis for better understanding the impacts of biological invasion on genetic configuration of local species.

Human activities and climate change have allowed many plant and animal species to recently expand their geographic ranges, a phenomenon likely to continue despite increasing quarantine efforts1. Impacts of biological invasions manifest at scales ranging from individuals to ecosystems and landscapes, potentially leading to evolutionary changes2,3. In the course of species’ expansion, the population dynamics and evolutionary processes of local flora and fauna may be affected4–6. Many factors, including genetic structure and variation in local populations, determine the capacity of native species to interact and adapt to the invader5. Higher trophic level species, such as parasitoids, in a given location may switch among taxonomically disparate but ecologically similar types of hosts by ecological sorting7, resulting in new associations or assemblages of related species8.

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a brassica-specialist herbivore of global significance9–12. It has achieved wide distribution across the world (i.e. East Asia and Oceania) in recent centuries, most likely due to human activities, such as globalization of trade and brassica crop cultivation10,13,14. In recent studies of *P. xylostella*, genetic homogeneity has been found in many populations across Asia-Pacific regions15–17. Insect movement due to air currents and transportation of agricultural products have been proposed as the main reasons for high levels of gene flow for *P. xylostella*18–20.

A broad range of natural enemies, including parasitoids, arthropod predators, pathogenic fungi and bacteria, attack *P. xylostella*21–23. Among these, *Cotesia vestalis (=plutellae)* Haliday (Hymenoptera: Braconidae) is one of

---

1State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Institute of Applied Ecology, Fujian Agriculture and Forestry University, Fuzhou, 350002, China. 2Joint International Research Laboratory of Ecological Pest Control, Ministry of Education, Fuzhou, 350002, China. 3Key Laboratory of Integrated Pest Management for Fujian-Taiwan Crops, Ministry of Agriculture, Fuzhou, 350002, China. 4Department of Biological Sciences, Brock University, 1812 Sir Isaac Brock Way, St. Catharines, Ontario, L2S 3A1, Canada. 5Graham Centre, Charles Sturt University, Orange, NSW, 2800, Australia. Correspondence and requests for materials should be addressed to S.Y. (email: sjyou@fafu.edu.cn) or M.Y. (email: msyou@fafu.edu.cn)
its most important biocontrol agents occurring in 38 countries occurring in 38 countries. Whilst C. vestalis has been introduced to Australia, North America, and the Caribbean in over 20 classical biological control programs, there are no records of it being introduced to Japan, Vietnam, Malaysia (Cameron Highlands) and China. Yet, C. vestalis is reported to be among the most predominant parasitoids of P. xylostella across parts of East Asia, and most likely associated to its tolerance to high temperatures and insecticides, and suggesting it may be native to this region.

The evolutionary success and diversification of insects has been aided, to some extent, by their complex associations with microorganisms. Over the recent decades, Wolbachia symbionts have attracted intensive research effort due to their diverse behavioral effects in a broad range of insect hosts, ability to manipulate the host reproductive system, and potential role in biological control of pests. Wolbachia are generally assumed to be maternally inheritable, with vertical transfer from egg cytoplasm to offspring, though recent studies have demonstrated horizontal transfer from infected to uninfected species.

Invasive species may change distribution, genetic configuration and trophic interactions of other native species and trigger rapid adaptation, which is increasingly becoming a major focus of research in evolutionary biology. In the present study, using samples of P. xylostella and C. vestalis collected across Asia countries of China, Nepal, Thailand, Malaysia and Vietnam, we used a set of mitochondrial genes to analyze the phylogeographic relationships to reveal the genetic diversity and demographic history of the two species; identify the geographic origin of C. vestalis; and adapt the adaptation of C. vestalis to the invasive P. xylostella in this region. Considering the Wolbachia arthropod associations and the potential role in intra-specific interactions, we also investigated the impacts of horizontal transfer of Wolbachia on the genetic configuration and co-evolution of local assemblages.

Results
Genetic diversity. A total of 1,621 bp DNA was obtained from concatenation of three P. xylostella mitochondrial genes (hereafter referred as p3m). From 323 P. xylostella individuals coming from 29 sampling locations (Table 1), we found 187 polymorphic sites and 212 haplotypes, representing a high haplotype diversity with an average of 0.931. We identified 174 haplotypes represented by single individuals, with the remainders represented by multiple P. xylostella. Nucleotide diversity was overall low with an average of 0.329%, except for the samples from three locations of Quanzhou in China (FJQZ, 0.606%), Cameron Highlands in Malaysia (MLCH, 0.906%) and Katmandu in Nepal (NPKT, 0.682%) (Table 1).

For C. vestalis, based on 1,232 bp DNA from three mitochondrial genes (hereafter referred as c3m), we found a relatively low haplotype and nucleotide diversity with an average of 0.415 and 0.172%, respectively. From 324 individuals, 43 polymorphic loci and 29 haplotypes were identified, with 19 haplotypes coming separately from single individuals and the rest from multiple individuals. We also observed higher haplotype and nucleotide diversity from two sampling locations of Guiyang (GZGY: Hd = 0.667 and θ = 0.431%) and Yuxi (YNXY: Hd = 0.769 and θ = 0.795%) in Southwest China (Table 1).

Phylogeny and haplotype network. The p3m-based phylogenetic tree revealed overall low genetic differentiation among individuals in terms of the branch length, and no isolated clusters containing individuals from specific geographic regions or populations, regardless of high genetic differentiation identified between Wolbachia PlutWB1-infected and -uninfected individuals (Fig. 1a). The Cytb-based network exhibited a star-like shape with many unique haplotypes present at the terminals (Fig. 1b), indicating recent population expansion by P. xylostella. In terms of this Cytb-based network, haplotype 2 (H2) was dominant and present in most sampled populations. The frequency of identified haplotypes was randomly distributed in each of the P. xylostella populations. The c3m-based phylogenetic tree (Fig. 2a) and haplotype network (Fig. 2b) showed four separate lineages with multiple basal clusters/lineages containing the samples from Southwest China. Lineage 1 comprised the samples from part of China, Malaysia and Vietnam, Lineage 2 consisted of individuals from China, Thailand and Nepal, while lineage 3 was represented by individuals from China only. Using the Cox1 gene sequences of our C. vestalis samples and additional sequences from India, Kenya, Benin, Hungary, Malaysia, New Zealand and Russia, we constructed a Cox1-based phylogeny (Fig. 2c) that does not change the overall c3m-based topology of the basal position and paraphyly of samples from Southwest of China (Fig. 2a,b).

Four primary lineages were present on the Wsp-based Phylogeny of Wolbachia (Fig. 3). Hosts involved in lineage 1 and 2 are C. vestalis (Lineage 1) and P. xylostella (Lineage 2), respectively, while lineage 4 was identified in both of these two species (Fig. 3).

Demographic history. Neutrality tests for P. xylostella were conducted using Tajima’s D and Fu’s Fs statistics (Table 1). The p3m-based Tajima’s D and Fu’s Fs statistics were significantly negative (Tajima’s D = −2.501, P < 0.001; Fu’s Fs = −5.532, P < 0.05) when all sampled populations were considered as one group. A significantly negative Tajima’s D value was associated with a significantly negative Fu’s Fs value, suggesting a recent expansion of P. xylostella populations in this region. The p3m-based mismatch distribution was unimodal when all sampled individuals of P. xylostella were considered as one group for analysis (Fig. 4a1), further supporting the concept of a recent expansion of the P. xylostella populations in East Asia.

Based on the neutrality tests for C. vestalis, c3m-based Tajima’s D and Fu’s Fs statistics also had significantly negative values (Tajima’s D = −1.747, P < 0.05; Fu’s Fs = −10.475, P < 0.001) when all sampled individuals taken as one group. In the defined clusters, only Lineage 1 showed significantly negative values of Tajima’s D (D = −2.323, P < 0.01) and Fu’s Fs (Fs = −23.426, P < 0.001), suggesting a recent expansion event of the C. vestalis populations. No population expansion events could be inferred in Lineage 2 (Tajima’s D = −1.421, P > 0.05; and Fu’s Fs = −3.066, (0.01 < P < 0.05)) and Lineage 3 (Tajima’s D = −1.133, P > 0.05; and Fu’s Fs = −1.362, P > 0.05). The c3m-based mismatch distribution was multimodal when all sampled individuals were considered as one group, but the three defined lineages exhibited unimodal distributions (Fig. 4a2–a5).
The paraphyly nature of samples from Southwest China, located at the early branching nodes of both c3m-based (Fig. 2a) and CoxI-based (Fig. 2c) phylogeny, suggests that Southwest China is the geographical origin of C. vestalis. Such an inference was also supported by the high nucleotide polymorphism of C. vestalis populations (YNXY and GZGY) in Southwest China (Table 1). We also found that all our sampled C. vestalis individuals derived from Southwest China based on the c3m-based phylogenetic tree (Fig. 2a) and haplotype network (Fig. 2b). According to the CoxI-based global phylogeny of C. vestalis (Fig. 2c, and supported by c3m tree, Fig. 2b), C. vestalis individuals from African, Oceanian and European countries were evolutionarily closely related to those from Thailand (TLPH) and Nepal (NPKT).

C. vestalis is one of the most common parasitic wasps of P. xylostella\(^{10}\) and previously considered to be native in Malaysia\(^{14}\), Japan\(^{20}\), and China\(^{25}\). It has also been suggested to originate from Europe based on the species description using Ukrainian specimens\(^{15}\). However, our Cox I-based phylogenetic analysis demonstrated that C. vestalis populations in Europe, Africa and Oceania derived from East Asia (Fig. 2b). Although no samples from the New World were included in this study, we speculate that the haplotypes from the New World may derive from the haplotypes of the Old World as C. vestalis was reported to be recently introduced into North America\(^{22,25}\) and South America\(^{23,24}\) as a biological control agent.

P. xylostella was recorded to colonize many regions (including East Asia) of the world in recent centuries\(^{14,15}\). A significantly negative Tajima’s D value was associated with a significantly negative Fu’s Fs value, suggesting a recent expansion of P. xylostella populations in East Asia, which is further supported by the unimodal p3m-based mismatch.
distribution of all sampled individuals. Our metadata analysis revealed a regional expansion of *C. vestalis* populations associated with the invasion and colonization of *P. xylostella* in East Asia. A comparable observation has also been reported for two *Diadegma* parasitoids of *P. xylostella* in Europe. The results of BEAST analysis also showed that the most recent common ancestor (TMRCA) of *C. vestalis* was between the TMRCA of *P. xylostella* in Old World (OW) and Oceania (OC), suggesting that the population expansion of *C. vestalis* could be related to the regional invasion of *P. xylostella* into Oceania through East Asia. Based on our results, we propose that the regional distribution of *C. vestalis* is a case of ecological sorting that adds *P. xylostella* to the hosts for *C. vestalis*. Such a new trophic association may facilitate the rapid adaptation of *C. vestalis* through altered life history traits and physiological manipulation.

The co-occurrence of *PlutWB1* in *P. xylostella* and *C. vestalis* (Lineage 4 in Fig. 3) suggested horizontal transfer of *Wolbachia* between herbivore and parasitoid. Our observation of *Wolbachia*-infected *P. xylostella* pupae and adults implied that the direction of this horizontal transfer was from *P. xylostella* to *C. vestalis*, given that *C. vestalis* would kill *P. xylostella* before pupation. In addition, in concordance with findings of Delgado & Cook, a distinct clade of five individuals (from different sampling locations) infected by *PlutWB1* were identified in the phylogenetic tree of *P. xylostella* (Fig. 1a), suggesting a long history of co-evolution between *P. xylostella* and this *Wolbachia* strain and a horizontal transfer scenario from the herbivore to its parasitoid. Another distinct clade (Lineage 3) in the *wsp*-based phylogenetic tree showed that the host of *Wolbachia* involved parasitoids, herbivores and predators, suggesting that the horizontal transfer of *Wolbachia* can occur across multiple trophic levels.

Li et al. reported that *Bemisia tabaci*-associated *Wolbachia* can be horizontally transferred between infected and uninfected individuals via plants. This can be further supported by a recent study of flowers and wild megachild bees, in which the plants can act as hubs for bacterial transmission between multiple organisms. In the present study, using our *wsp* sequences and the NCBI-based *wsp* sequences, we found that the individuals infected by *Wolbachia* were involved with several species of herbivores (Lineage 3 in Fig. 3). We assumed that the presence of such a *Wolbachia* lineage (Lineage 3) in various herbivores might have come from food intake or potentially from horizontal transfer between the species.
In this study, using an interactive system involving an invasive herbivore, *P. xylostella*, and its parasitoid, *C. vestalis*, we demonstrated how the invasion of an alien host herbivore (*P. xylostella*) could significantly affect the genetic variation of a higher trophic species (*C. vestalis*). Through ecological sorting, this parasitoid could have switched from an original local host to *P. xylostella* and, as an important agent of classical biological control, it underwent significant, human-aided, population expansion. In addition, during this expansion, the endosymbiont *Wolbachia* (*plutWB1*) in *P. xylostella* was also introduced into local species. Our work provides a comprehensive picture (Fig. 5) of how invasion by an alien species can trigger significant evolutionary changes in a newly associated parasitoid and the transmission of exotic bacteria into local species, leading to the formation of new biological interactions and the genetic configuration of local species.

**Methods**

**Sample collection and species identification.** We collected *P. xylostella* and *C. vestalis* samples from the same or nearby cabbage and broccoli fields in China (25), Nepal (1), Thailand (1), Vietnam (1) and Malaysia (2) from 2012 to 2014 (Fig. S1; Table S1). Twenty-eight samples of *P. xylostella* and *C. vestalis* were collected from common locations, and one sample of *P. xylostella* was collected in Chongqing, China (CQ) and its counterpart *C. vestalis* sample from nearby, Luzhou in Sichuan Province, China (SCLZ) (Fig. S1; Table S1). The second and third instar larvae of *P. xylostella* were maintained on vegetable leaves for emergence of parasitoids. *P. xylostella* pupae and adults, and *C. vestalis* cocoons were morphologically identified and preserved in 95% ethanol. Specimens were stored at −80 °C prior to DNA extraction. A total of 323 *P. xylostella* and 324 *C. vestalis* individuals were used in this study. We used a 600 bp mitochondrial gene sequence (CoxI) (Table S2) and DNA barcoding criteria to individually confirm the species identity of *P. xylostella* and *C. vestalis* based on BOLD.[44]

**DNA Extraction and sequencing.** Total genomic DNA was extracted from individual insects using DNeasy Blood and Tissue Kit (Qiagen, Germany). Three mitochondrial genes, CoxI, cytochrome b (Cytb), and NADH dehydrogenase subunit I (NadhI) were sequenced for both *P. xylostella* and *C. vestalis* (Table S2). Primers were developed for *Cytb* and *NadhI* of *P. xylostella* as well as *Cytb* and *NadhI* of *C. vestalis* using Primer Premier version 5 (Premier Biosoft International, Palo Alto, CA, USA) based on the reference mitochondrial genomes. Primers of other gene segments were referred to published references (Table S2).
PCR was conducted using the Mastercycler pro system (Eppendorf, Germany) under the following conditions: an initial denaturation for 2 min at 94 °C, followed by 35 cycles of 10 s at 96 °C, 15 s at specific annealing temperature of each genes (Tables S2), and 1 min at 72 °C, and a subsequent final extension for 10 min at 72 °C. Amplified products were purified and bidirectionally sequenced using the ABI 3730xl DNA Analyzer by Sanboyuanzhi Biotechnology Co., Ltd. (Beijing, China). All the sequences were deposited in Genebank database (accession number from KX604356 to KX606864).

Infection of *P. xylostella* and *C. vestalis* by Wolbachia was determined using ∼600 bp products of the *wsp* gene amplified with specific primers (Table S2). A positive control of PCR reaction (with DNA of *Wolbachia* infected samples as templates) was used to test infection of *Wolbachia* in samples.

**Genetic analysis.** Sequences for each of the gene fragments were aligned using MEGA5.2. All mitochondrial sequences for each of the individuals of both insect species were aligned independently using MAFFT-7.037. Conservative regions selected by Gblock-0.91b were used for gene concatenation, which was performed by Sequence-Matrix-1.7.8 with default parameters. Parameters of genetic diversity, haplotype diversity (Hd) and nucleotide diversity (θ), were calculated using the DnaSpV5. Populations with <5 individuals (3 populations of *P. xylostella* and 12 of *C. vestalis*) were not included in the calculation of the parameters related to the genetic diversity (Table 1).
Phylogenetic and network analysis. Phylogeographic analysis can be used to explore the evolutionary history of a species or to examine the temporal and spatial effects on co-evolutionary relationships of closely related species. This type of study can help reveal the impacts of biological expansions on local communities over wide spatial scales. Using the sequences of three concatenated mitochondrial genes, phylogenetic relationships were constructed for *P. xylostella* (hereafter p3m) and *C. vestalis* (hereafter c3m). We selected *Cotesia flapvis*, which is from the *Cotesia* genus, as the outgroup for *C. vestalis* phylogenetic tree construction. The *CoxI*
sequences of *C. vestalis* and *C. flapivis* (outgroup) from NCBI (http://www.ncbi.nlm.nih.gov/) were also downloaded for construction of a global phylogenetic tree of *C. vestalis*. The phylogeny of wsp gene was developed using the NCBI-based wsp sequences with the best hit (with <3 gaps and > = 99% identity) when BLAST conducted using the wsp sequences from this study, plus additional sequences of the wsp gene of *Wolbachia* parasitizing *P. xylostella* and *C. vestalis*. Phylogenetic inferences were performed using the neighbor-joining (NJ) and maximum likelihood (ML) methods by PAUP*4.0b10*. The software MrModeltest version 2.36 was used to select the best-fit nucleotide substitution model. The General Time Reversible model was used with invariable sites and a gamma-shaped distribution of rates across sites (GTR + I + G) based on the Akaikes Information Criterion (AIC).

The network analysis was conducted for mitochondrial genes of *P. xylostella* and *C. vestalis* using median-joining algorithm implemented in the software Network, version 4.6.1.37. We constructed the haplotype networks of both species for individual genes as well as the concatenated mitochondrial gene sequences. Haplotype type and frequency for each population were also recorded.

**Demographic analysis.** We calculated the Tajima’s *D* and Fu’s *Fs* for each of the populations (≥5 individuals) of the two species based on the concatenated mitochondrial genes using DnaSP V5. Analyses of mismatch distributions were also performed for both species. For *C. vestalis*, mismatch distribution was analyzed for not only a collection of all samples, but also three major clusters based on the phylogenetic tree of three concatenated mitochondrial genes.

BEAST18 was also used to calculate the coalescent time of lineages in *P. xylostella* and *C. vestalis*, based on Coxi sequences, which has a reported mutation rate. For *P. xylostella*, more samples from the Old World and Oceania were included to increase precision in coalescent time inference. As the mutation rates varied among insect lineages, we used lognormal relaxed clock while estimating the evolutionary timescales. The chain of Markov Chain Monte Carlo (MCMC) was set to 50 million with Log parameters in every 5000.

**Data Availability** Data generated during the study available in Genbank with the primary accession number from KX604356 to KX606864.

**References**

1. Bebber, D. P. Range-expanding pests and pathogens in a warming world. *Annu. Rev. Phytopathol.* 53, 335, https://doi.org/10.1146/annurev-phyto-080614-120207 (2015).
2. Pejchar, L. & Mooney, H. A. Invasive species, ecosystem services and human well-being. *Trends Ecol. Evol.* 24, 497–504, https://doi.org/10.1016/j.tree.2009.03.016 (2009).
3. Ehrenfeld, J. G. Ecosystem consequences of biological invasions. *Annu. Rev. Ecol. Evol. S.* 41, 59–80, https://doi.org/10.1146/annurev-ecolsys-102209-144650 (2010).
4. Bezemer, T. M., Harvey, J. A. & Cronin, J. T. Response of native insect communities to invasive plants. *Annu. Rev. Entomol.* 59, 119, https://doi.org/10.1146/annurev-ento-011613-162104 (2014).
5. Strauss, S. Y., Lau, J. A. & Carroll, S. P. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecol. Lett.* 9, 357–374, https://doi.org/10.1111/j.1461-0248.2005.00874.x (2006).
6. Pintor, L. M. & Byers, J. E. Do native predators benefit from non-native prey? *Ecol. Lett.* 18, 1174–1180, https://doi.org/10.1111/ele.12496 (2015).
7. Weiher, E. & Keddy, P. Ecological assembly rules: perspectives, advances, retreats. (Cambridge University Press, 2001).
8. Althoff, D. M. A test of host-associated differentiation across the ‘parasite continuum’ in the tri-trophic interaction among yuccas, *Agave americana*, and its associated insects. *Mol. Ecol.* 21, 1385–1397, https://doi.org/10.1111/j.1365-294X.2012.05632.x (2012).
9. Li, Z., Feng, X., Liu, S., You, M. & Furlong, M. J. Biological ecology, and management of the diamondback moth in China. *Annu. Rev. Entomol.* 61, 277–296, https://doi.org/10.1146/annurev-ento-011715-041801 (2016).
10. Furlong, M. J., Wright, D. J. & Doddall, L. M. Diamondback moth ecology and management: problems, progress, and prospects. *Annu. Rev. Entomol.* 58, 517–541, https://doi.org/10.1146/annurev-ento-120811-153605 (2013).
11. Talekar, N. S. & Shelton, A. M. Biology, ecology, and management of the diamondback moth. *Annu. Rev. Entomol.* 38, 275–301, https://doi.org/10.1146/annurev.ento.38.010193.001423 (1993).
12. You, M. et al. A heterozygous moth genome provides insights into herbivory and detoxification. *Nat. Genet.* 45, 220, https://doi.org/10.1038/ng.2524 (2013).
13. Kfir, R. Origin of the diamondback moth (Lepidoptera: Plutellidae). *Ann. Entomol. Soc. Am.* 91, 164–167, https://doi.org/10.1017/S0007485316000766 (1998).
14. Horii, K. & Shiraki, T. Investigation of pest insect in Taiwan. In Special Report Formosa Agricultural Experiment Station 228 (1910).
15. Wei, S. et al. Genetic structure and demographic history reveal migration of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) from the southern to northern regions of China. *PloS One* 8, e63125, https://doi.org/10.1371/journal.pone.0063125 (2013).
16. Enderby, N. M., McKechnie, S. W., Ridland, P. M. & Weeks, A. R. Microsatellites reveal a lack of structure in Australian populations of the diamondback moth, *Plutella xylostella* (L.). *Mol. Ecol.* 15, 107–118, https://doi.org/10.1111/j.1365-294X.2005.02789.x (2006).
17. Yang, J. et al. Insight into the migration routes of *Plutella xylostella* in China using miCOI and ISSR markers. *PloS One* 10, e0130905, https://doi.org/10.1371/journal.pone.0130905 (2015).
18. Niu, Y. Q., Nansen, C., Li, X. W. & Liu, T. X. Geographical variation of *Plutella xylostella* (Lepidoptera: Plutellidae) populations revealed by mitochondrial COI gene in China. *J. Appl. Entomol.* 138, 692–700, https://doi.org/10.1111/jen.12130 (2015).
19. Fu, X., Xing, Z., Liu, Z., Ali, A. & Wu, K. Migration of diamondback moth, *Plutella xylostella*, across the Bohai Sea in northern China. *Crop Prot.* 64, 143–149, https://doi.org/10.1016/j.cropro.2014.06.021 (2014).
20. Ke, E. et al. Genetic differentiation of the regional *Plutella xylostella* populations across the Taiwan Strait based on identification of microsatellite markers. *Ecol. Evol.* 5, 5880–5891, https://doi.org/10.1002/ece3.1850 (2015).
21. Delvare, G. The taxonomic status and role of Hymenoptera in biological control of DBM, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). In *Improving biological control of Plutella xylostella* (eds Kirk, A. A. & Bordat, D.) 17–49 (CIRAD, Montpellier, France, 2004).
22. Shelton, A. M. A brief review of diamondback moth biological control in North America. In *Improving biological control of Plutella xylostella* (eds Kirk, A. A. & Bordat, D.) 93–102 (CIRAD, Montpellier, France, 2004).
23. Alvi, M. S. & Momoi, S. Environmental regulation and geographical adaptation of diapause in *Cotesia plutellae* (Hymenoptera: Braconidae), a parasitoid of the diamondback moth larvae. *Appl. Entomol. Zool.* 29, 4374–4380, https://doi.org/10.1303/aez.29.89 (1994).
24. Ooi, P. A. Role of parasitoids in managing diamondback moth in the Cameron Highlands, Malaysia. In Diamondback moth and other crucifer pests: proceedings of the Second International Workshop, 255–262 (AVRDC, Tainan, Taiwan, 1992).
25. Liu, S., Wang, X., Guo, S., He, J. & Shi, Z. Seasonal abundance of the parasitoid complex associated with the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae) in Hangzhou, China. B. Entomol. Res. 90, 221–231, https://doi.org/10.1017/S0007463500003841 (2000).
26. Shi, Z. & Liu, S. Interspecific interactions between Cotesia plutellae and Oomyzus sokolowiski, two major parasitoids of diamondback moth, Plutella xylostella. J. Appl. Ecol. 14, 949–954, https://doi.org/10.1111/j.1365-2664.2003.00869.x (2003).
27. Shi, Z. H., Li, Q. B. & Li, X. Interspecific competition between Diadegma semiclausum Hellen (Hym., Ichneumonidae) and Cotesia plutellae (Kurdjumov) (Hymenoptera: Braconidae) in parasitizing Plutella xylostella (L.) (Lepidoptera: Plutellidae). J. Appl. Entomol. 128, 437–444 (2004).
28. Verkerk, R. & Wright, D. J. Field-based studies with the diamondback moth tritrophic system in Cameron Highlands of Malaysia: implications for pest management. Int. J. Pest Manage. 43, 27–33, https://doi.org/10.1080/096708797289414 (1997).
29. Visser, A. A., Nore, T., Currie, C. R., Aanen, D. K. & Poulsen, M. Exploring the potential for actinobacteria as defensive symbionts in fungus-growing termites. Microbiol. Ecol. 63, 975–985, https://doi.org/10.1007/s00248-011-9987-4 (2012).
30. Salem, H., Kreutzler, E., Sudakaran, S. & Kaltenpoth, M. Actinobacteria as essential symbionts in firebugs and cotton seeders (Hemiptera: Pyrrhocoridae). Environ. Microbiol. 15, 1956–1968, https://doi.org/10.1111/j.1462-2920.2013.021013 (2013).
31. Werren, J. H., Baldo, L. & Clark, M. E. Wolbachia: master manipulators of invertebrate biology. Nat. Rev. Microbiol. 6, 741–51, https://doi.org/10.1038/nrmicro1969 (2008).
32. Kawai, S., Juric, I., Salzburger, W. & Balmer, O. Spread and global population structure of the diamondback moth in China, and Grant FT140101303 in Australia. No. 31230061), Fujian-Taiwan Joint Innovation Centre for Ecological Control of Crop Pests, the “111” Program in China, and Grant FT140101303 in Australia.
33. Stuart, Y. E. et al. Rapid evolution of a native species following invasion by a congener. Science 346, 463–6, https://doi.org/10.1126/science.1257088 (2014).
34. McFrederick, Q. S. et al. Role of parasitoids in managing diamondback moth in the Cameron Highlands, Malaysia. In Diamondback moth and other crucifer pests: proceedings of the Second International Workshop, 255–262 (AVRDC, Tainan, Taiwan, 1992).
35. McFrederick, Q. S. et al. Flowers and wild megachilid bees share microbes. Microb. Ecol. 73, 188–200, https://doi.org/10.1007/s00248-016-0838-1 (2017).
36. Ratnasingham, S. & Hebert, P. D. BOLD: The barcode of life data system (http://www.barcodinglife.org). Mol. Ecol. Notes 7, 355–364, https://doi.org/10.1111/j.1471-2229.2007.01678.x (2007).
37. Tamura, K. et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739, https://doi.org/10.1093/molbev/msr012 (2011).
38. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780, https://doi.org/10.1093/molev/mlt010 (2013).
39. Talavera, G. & Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biol. 56, 564–577, https://doi.org/10.1093/sysbio/sym014 (2007).
40. Vaidya, G., Lohman, D. J. & Meier, R. SequenceMatrice: concatenation software for the fast assembly of multi-genus datasets with character set and codon information. Cladistics 27, 171–180, https://doi.org/10.1111/j.1095-8399.2010.01032.x (2011).
41. Librado, P. & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1415–1415, https://doi.org/10.1093/bioinformatics/btp187 (2009).
42. Sproul, J. S. et al. Comparative phylogeography of codistributed aquatic insects in western North America: insights into dispersal and regional patterns of genetic structure. Freshwater Biol. 59, 2051–2063, https://doi.org/10.1111/fwb.12406 (2014).
43. Oliveira, M. R. C., Correia, A. S., de Souza, G. A., Guedes, R. N. C. & de Oliveira, L. O. Mesoamerican origin and pre-and post-Columbian expansions of the ranges of Acanthoscelides obtectus Say, a cosmopolitan insect pest of the common bean. PLoS One 8, e70039, https://doi.org/10.1371/journal.pone.0070039 (2013).
44. Rewicz, T., Wattier, R., Grabowski, M., Rigaud, T. & Bącela-Spychalska, K. Out of the Black Sea: phylogeography of the invasive killer shrimp Dikerogammarus villosus across Europe. PLoS One 10, e0118121, https://doi.org/10.1371/journal.pone.0118121 (2015).
45. Papadopoulo, A., Anastasiou, I. & Vogler, A. P. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. Mol. Biol. Evol. 27, 1659–1672, https://doi.org/10.1093/molbev/msq251 (2010).
46. Nicholls, J. A. et al. Concordant phylogeography and cryptic speciation in two Western Palaearctic oak gall parasitoid species complexes. Mol. Ecol. 19, 592–609, https://doi.org/10.1111/j.1365-294X.2009.04499.x (2010).
47. Swofford, D. L. PAUP*: phylogenetic analysis using parsimony, version 4.0 b10. (2003).
48. Nylander, J. MrModeltest v2. Program distributed by the author. 2004. Evolutionary Biology Centre, Uppsala University (2009).
49. Bandelt, H. J., Forster, P. & Röhl, A. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48, https://doi.org/10.1093/oxfordjournals.molbev.a020836 (1999).
50. Drummond, A. J. & Rambaut, A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214, https://doi.org/10.1186/1471-2148-7-214 (2007).

Acknowledgements
This work was financially supported by the National Natural Science Foundation of China (No. 31320103922 and No. 31230061), Fujian-Taiwan Joint Innovation Centre for Ecological Control of Crop Pests, the “111” Program in China, and Grant FT140101303 in Australia.
Author Contributions
F.K., S.Y., S.H., M.Y. and W.H. conceived, designed and/or managed the project. S.H., W.C., F.K., T.L., D.X., Q.L. and X.L. collected insects and/or prepared DNA samples for sequencing. F.K., S.Y., S.H., M.Y., L.V. and G.M.G. performed data analyses, co-wrote the manuscript, and interpreted results and/or revised the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-46742-3.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019