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Lack of Genetic Structure Among Populations of Striped Flea Beetle *Phyllotreta striolata* (Coleoptera: Chrysomelidae) Across Southern China

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The striped flea beetle (SFB) *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae) is a major pest of cruciferous vegetables in southern China. The population diversity and genetic structure of SFB are unknown. Here, we assembled a draft genome for the SFB and characterized the distribution of microsatellites. Then, we developed 12 novel microsatellite markers across the genome. We used a segment of the *cox1* gene and newly developed microsatellite markers to genotype the genetic diversity of SFB across southern China. There were 44 mitochondrial haplotypes in the SFB populations, with haplotype 2 as the most widespread. The population genetic differentiation was very low, indicated by F_{ST}-values (<0.05 except for Guangxi population with other populations based on *cox1*), high gene flow (4.10 and 44.88 of *cox1* and microsatellite, respectively) and Principal Coordinate Analysis across all populations. Mantel test showed genetic distance in SFB was significantly associated with geographic distance based on microsatellites (R^2 = 0.2373, P = 0.014) while result based on *cox1* (R^2 = 0.0365, P = 0.155) showed no significant difference. The phylogenetic analysis did not find any geographically related clades among all haplotypes. Analyses based on microsatellites showed a lack of population genetic structure among all populations. Our study provides a foundation for the future understanding of the ecology and evolution of SFB and its management.

Keywords: *Phyllotreta striolata*, southern China, population genetic structure, microsatellite, mitochondrial gene

INTRODUCTION

The striped flea beetle (SFB) *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae) is a pest of cruciferous crops (Brassicaceae) (Soroka et al., 2018; Cao et al., 2020; Atirach et al., 2021). The adults chew on leaves and larvae feed on fiber. The field control of this pest heavily relys on insecticides (Andersen et al., 2006), leading to insecticide resistance (Feng et al., 2000; James et al., 2019).

The SFB is mainly distributed in Asia, Europe, and North America (Soroka et al., 2018; Cao et al., 2020; Atirach et al., 2021). Native to Eurasia, SFB was introduced to North America in 1663 from Europe (Rousseau and Lesage, 2016). In China, the SFB is one of the most important cruciferous crops pests in southern areas. When the adult density of SFB is 20∼50 individuals every 100 *Brassica pekinensis*, the damage rate will be 50∼76%; if the adult density more than 50, the damage rate will be 100% in Shenzhen (Zhang et al., 2000). There are six to nine generations of SFB throughout the year without overwintering in southern China (Sun et al., 2010), compared to one to two generations per year in North America (Olfert et al., 2017). Recently, damage of SFB has increased. Its geographic range expanded both in China and North America (Chai, 2010; Sun, 2010; Lee et al., 2011; Kielen, 2012). Current studies mainly focus on this pest's biology and control methods (Gao et al., 2000; Chai, 2010; Lee et al., 2011; Soroka et al., 2018; Yan et al., 2018; Atirach et al., 2021); however, no studies that we are aware of on the ecology and evolution of the SFB.

In this study, we examined the population genetic diversity and genetic structure of the SFB among populations across its main distribution range in China. Due to the lack of genetic markers, we developed a novel set of microsatellite markers from first-time assembled random genomic sequences of SFB using Illumina sequencing. We used both microsatellites and a segment of the mitochondrial cytochrome oxidase subunit I (*cox1*) gene to determine the genetic diversity and differentiation among eight representative populations across southern China. We tested the hypothesis that levels of genetic diversity and population differentiation for the SFB are low due to high levels of gene flow among populations. These results provide a basis to understand the ecology and evolution of this pest and its management.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Adults of SFB were collected across southern China from cruciferous vegetables (**Table 1** and **Figure 1**). To avoid collecting siblings, we collected specimens from about 20 sites at least 10 m apart at each sampling location. In total, eight populations were collected. The species were first identified by morphology (He et al., 2012) and then validated by molecular identification using the mitochondrial *cox1* gene (see below). All samples were kept in 100% ethanol before DNA extraction.

To construct a high-throughput sequencing library for microsatellites development, we extracted genomic DNA from 50 adults collected in Xiaoshan, Zhejiang province, using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Then, we used 192 individuals for population-level genotyping, with 24 individuals from each population. Total genomic DNA was extracted from individual whole adult using the DNeasy Blood & Tissue Kit. The voucher specimens were stored at –80°C in the Integrated Pest Management Laboratory of the Beijing Academy of Agriculture and Forestry Sciences.

Library Construction, Genome Sequencing, and Assembly

The high-throughput sequencing library with 500-bp insert size was prepared using the Illumina TruSeq DNA PCR-Free HT Library Prep Kit (Illumina, San Diego, CA, United States). The prepared library was sequenced on an Illumina Hiseq4000 Sequencer using the Hiseq Reagent Kit v3 (Illumina, San Diego, CA, United States) by Beijing BerryGenomics Co., Ltd. The paired-end 150 bp raw data were trimmed by removing the low-quality reads using Trimmomatic 0.36 (Bolger et al., 2014), and then the sequences were evaluated by FastQC v 0.11.5 (Andrews, 2004). The genome size of *P. striolata* was estimated by JELLYFISH v2.2.6 software with a K-mer method (Kingsford, 2011). IDBA_UD v1.1.1 was used to assemble the generated genomic sequences with K-mer from 20 to 140 (Peng et al., 2010).

Development of Universal Microsatellite Markers

The Microsatellite Search and Building Database (MSDB)[1] was used to identify potential microsatellite sites from the whole genome sequences of *P. striolata*. A minimum of 12, 5, 5, 5, 5, and 5 repeats was used to distinguish mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide motifs, respectively (Lianming et al., 2013). The QDD3 program (Emese et al., 2010) was then used to extract microsatellites and their flanking sequences (300 bp each) and design the primers. The criteria and parameters for the primer design for the isolated microsatellite markers followed Cao et al. (2016). The primer design was set according to the following parameters: annealing temperature (Tm): 54°C < Tm < 60°C; the difference in Tm between paired upstream and downstream primers was < 4°C; only one pair of microsatellite primers is kept on each locus; the repeat motif of the amplified product was ≥ 3. The microsatellite loci were further filtered under stringent criteria as described in previous studies (Song et al., 2018): (i) the microsatellites had to be pure and specific, (ii) the design strategy of "A" was used, and (iii) the minimum distance between the 3′ end of a primer pair and its target region had to be no shorter than 10 bp.

We selected 70 pairs of primers for initial validation using one individual from each representative population (AH, FJ, GD, GX, HN, JS, JX, ZJ). We used three fluorescences (FAM, ROX, HEX) to label the amplified products of each locus (Schuelke, 2000; Blacket et al., 2012). The fluorescences were independently added to a primer C tail (PC-tail) (5′ CAGGACCAGGCTACCGTG 3′). The sequence of the PC-tail was added to the 5′ end of each upstream primer (Blacket et al., 2012). The reaction system was set to 10 μL, including 0.5 μL of template DNA (30–160 ng/μL), 5 μL of Master Mix (Promega, Madison, WI, United States), 0.08 μL of the PC tail modified forward primer (10 mM), 0.16 μL of reverse primer (10 mM), 0.32 μL of fluorescence-labeled PC-tail (10 mM) and 3.94 μL of ddH_2O. The following PCR amplification program was used to amplify the microsatellites: 3 min at 94°C; 35 cycles of 15 s at 94°C, 30 s at 56°C and 1 min at

[1]http://msdb.biosv.com/

TABLE 1 | Population information of *Phyllotreta striolata* used in this study.

Population	Collection location	Longitude (°E), Latitude (°N)	Collection date (month/year)	Number of haplotypes
AH	Xuanzhou, Xuancheng of Anhui province	118.89, 30.90	10/2020	8
FJ	Yunxiao, Zhangzhou Fujian province	117.58, 23.95	10/2020	4
GD	Gaoyao, Zhaoqing of Guangdong province	112.58, 23.05	10/2020	6
GX	Yizhou, Hechi of Guangxi province	108.83, 24.49	10/2020	6
HN	Xiuying, Haikou of Hainan province	110.25, 19.98	10/2020	7
JS	Huishan, Wuxi of Jiangsu province	120.24, 31.66	10/2020	6
JX	Shanggao, Yichun of Anhui province	114.90, 28.23	10/2020	4
ZJ	Xiaoshan, Hangzhou of Zhejiang province	120.63, 30.21	04/2016	3

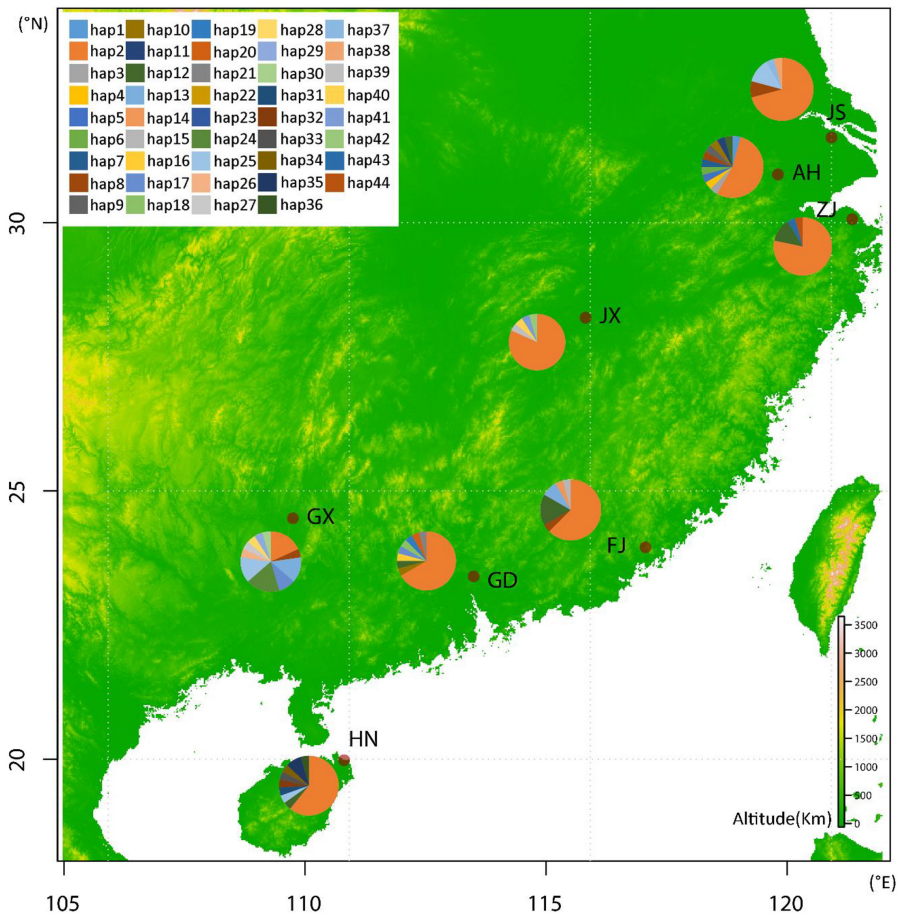

FIGURE 1 | Collection sites and geographical distribution of the mitochondrial *cox1* haplotypes of *Phyllotreta striolata*. The haplotype frequency for each population is represented by colors in the pie charts. See **Table 1** for population codes.

72°C, followed by a final 10 min extension at 72°C. Primer pairs fitting the following criteria were retained in subsequent analysis: (i) primers with PCR amplification rate equal or higher than 75%; (ii) at least two alleles present in tested individuals; (iii) without non-specific amplification.

Length of PCR products was analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems, United States) using the GeneScan^{TM} 500 LIZ^{TM} dye Size Standard (Applied Biosystems, United States) for sizing DNA fragments. Genotypes were determined using GENEMAPPER v4.0 (Applied Biosystems, United States) (Chatterji and Pachter, 2006). The remaining primer pairs were used for population-level genotyping.

Mitochondrial Gene Amplification, Sequencing, and Homologous Gene Downloading

To characterize the mitochondrial variation and validate correct identification of the specimens, a fragment of the *cox1* gene involved the DNA barcoding region of insects

was sequenced using the universal primers LCO1490 (5′ GGTCAACAAATCATAAAGATATTGG 3′) and HCO2198 (5′ TAAACTTCAGGGTGACCAAAAAATCA 3′) (Folmer et al., 1994). Polymerase chain reactions (PCR) were conducted using the Mastercycler Pro system (Eppendorf, Germany) in a 15 μL volume consisting of 1 μL of template DNA (30–160 ng/μL), 7.5 μL of Master Mix (Promega, Madison, WI, United States), 0.6 μL of forward primer (10 mM), 0.6 μL of reverse primer (10 mM) and 5.3 μL of ddH_2O. The thermal profiles for DNA amplification were as follows: 3 min at 94°C; 35 cycles of 15 s at 94°C, 20 s at 54°C, and 40 s at 72°C, followed by a final 10-min extension at 72°C. Amplified products were purified and sequenced on an ABI 3730xl DNA Analyzer by Tsingke Biotechnology Co. Ltd. (Beijing, China).

Apart from sequences obtained in this study, we searched orthologous *cox1* gene sequences from the NCBI nucleotide database and included those with sampling locations in our phylogenetic analysis (Pentinsaari et al., 2014; Hendrich et al., 2015; Nie et al., 2017; Coral şahin et al., 2018; Lalrinfeli et al., 2019) (**Supplementary Table 1**). Sequences with obvious errors were deleted, and others were used for follow-up analysis.

Population Genetic Diversity Analysis

For mitochondrial DNA, sequencing results from both strands were assembled using SeqMan in the LASERGENE version 7.1.2 (DNASTAR, Inc., United States). Sequences of the *cox1* were aligned with MUSCLE (Edgar, 2004) implemented in MEGA version X (Kumar et al., 2018). The number of polymorphic sites (S), number of haplotypes (H), haplotype diversity (H_d), nucleotide diversity (Pi), Tajima's *D*, Fu's F_s and gene flow (N_m) were analyzed in DnaSP version 6 (Rozas et al., 2017). Pairwise mean population differentiation (F_{ST}) was estimated using Arlequin 3.5 (Excoffier and Lischer, 2010).

For microsatellites, the number of alleles and the observed heterozygosity (H_o) were analyzed using the macros Microsatellite Tools (Park, 2001). The null allele frequencies and F_{ST} with excluding of null alleles (ENA) were estimated using the software FreeNA (Chapuis and Estoup, 2007). Deviation from Hardy-Weinberg equilibrium (HWE) for each locus/population combination, F_{ST} and inbreeding coefficients (F_{IS}) were estimated in GENEPOP v4.0.11 (Rousset, 2008). Principal Coordinate Analysis (PCoA) and genetic differentiation coefficient (G_{ST}) were performed with the GenAlex program ver. 6.502 (Peakall and Smouse, 2012). The gene flow was calculated using the formula $N_m = 0.5 (1- G_{ST})/G_{ST}$. HP-RARE v1.1 (Kalinowski, 2010) was used to test allelic richness (A_R) and allelic richness of private alleles (P_{AR}) of each site. GENCLONE v2.0 (Arnaud-Haond and Belkhir, 2006) was used to estimate the total number of alleles (A_T) and the unbiased expected heterozygosity (H_E). We compared the number of alleles (A_S) among samples with different sample sizes in GENCLONE. Mantel test (Mantel, 1967), used to estimate correlation between genetic and geographic distances of populations to test isolation by distance, was performed with 1,000 permutations based on Genepop.[2]

Phylogenetic and Population Genetic Structure Analysis

A Bayesian-inference phylogenetic tree was constructed for mitochondrial DNA using Mrbayes v3.2.2 (Ronquist et al., 2012) to examine phylogenetic relationships among *cox1* haplotypes of SFB. Four independent Markov chains for 50 million MCMC generations were run with tree sampling every 5,000 generations and a burn-in of 2,500 trees.

For microsatellite data, the population genetic structure was first investigated using the STRUCTURE v2.3.4 program (Pritchard et al., 2000). We used 30 replicates of each *K*-value from 1 to 8, with 200,000 Markov chain Monte Carlo iterations and a burn-in of 100,000 iterations. The results were uploaded to the online software Structure Harvester v0.6.94 (Earl and Vonholdt, 2012) to determine the optimal *K*-value by a Delta K method (last accessed on 28, Dec. 2020).[3] The *K*-value with the highest ΔK represents the number of potential genetic clusters in the population. Membership coefficient matrices (Q-matrices) associated with the optimal K were processed using CLUMPP v1.12 (Jakobsson and Rosenberg, 2007) and then visualized using the DISTRUCT v1.1 (Taubert et al., 2019). Finally, we used discriminant analysis of principal component (DAPC) to analyze population genetic structure under default settings to complement the STRUCTURE analysis. This analysis was run using an R-package adegenet v1.4-2 (Jombart et al., 2008).

RESULTS

Genome Assembly and Characterization of Microsatellites Across the Genome

A total of 47.88 Gb paired-end (PE) sequences (159,598,840 reads each with a length of 150 bp) were obtained. Trimmed reads were assembled into 595,192 scaffolds with a total length of 342.147 MB ranging from 200 bp to 118.198 KB with an N50 of 600 bp. These contigs were used for microsatellite discovery.

In total, 28,512 microsatellites were isolated from the randomly sequenced genome sequences of SFB with 8,635 mononucleotide repeat sites (30.29%), 6,735 (23.62%) dinucleotide repeat (DNR) sites, 8,115 (28.46%) trinucleotide repeat (TNR) sites, 3,550 (12.4%) tetranucleotide repeat (TTNR) sites, 905 (3.17%) pentanucleotide repeats (PNR) sites and 572 (2.00%) hexanucleotide repeat (HNR) sites. In decreased order, A, AC, AT, and AAT were the most frequent repeat motifs. The microsatellites with the three most frequently repeated motifs occupied 51.9% of all microsatellites. The average length of the repeat region ranged from 14.4 to 49.44 bp.

Development of Microsatellite Markers

The QDD3 program designed 2,585 primer pairs for 1,994 din-, 359 tri-, 70 tetra-, 29 penta-, and 21 hexa-nucleotide

[2]https://genepop.curtin.edu.au/

[3]http://taylor0.biology.ucla.edu/structureHarvester/

microsatellites. Following our stringent filter, 70 primer pairs flanking perfect microsatellites were retained. The number of primer pairs flanking tri-, tetra-and penta-nucleotide microsatellites was 67, 1, and 2, respectively. In our initial test of these 70 primer pairs, 16 pairs generated polymorphic genotypes, 12 pairs failed to amplify in any samples, and 46 pairs failed to amplify in more than two individuals. In the final test, 12 microsatellite markers were retained for population-level genotyping (**Table 2**).

Population Genetic Diversity

Based on the mitochondrial *cox1* genes which were 647 bp long, we found 44 haplotypes from the collected SFB populations. Haplotype #2 was the most common (61.17% of individuals). Thirty-four haplotypes appeared in only one population. The GX population had the most haplotypes with thirteen, while the ZJ population had the least number of haplotypes (n = 4). The haplotype diversity ranged from 0.338 to 0.931, and the nucleotide diversity ranged from 0.00059 to 0.00497. There were 11 polymorphic sites in the HN population which represented the highest number across the most in all populations (**Table 3**). The data of Tajima's D and Fu's F_s of each population were all negative except for F_S of GX (F_S = 0.57 without statistical significance). For all populations Tajima's D and Fu's F_s were –2.53 and –6.01, respectively, with statistical significance, which showed the SFB populations followed the neutral evolution model and there was a population expansion in recent history. The Nei's N_m of haplotypes and all sequences was 4.10 and 3.37, respectively.

Using all *cox1* sequences from the public database, haplotypes were reanalyzed and all populations were divided into thirty-four haplotypes. Haplotypes #30–34 were unique to populations outside China. Haplotype #2 was shared by populations in and out of China. The other twenty-eight haplotypes were unique to the populations of China. The pairwise distance among all haplotypes ranged from 0.0018 to 0.0143 (**Table 4**). We also calculated the pairwise population genetic differentiation among eight populations and F_{ST}-values ranged from –0.0187 (FJ-ZJ) to 0.1374 (JX-GX). A moderate genetic differentiation was observed between GX and other populations based on the F_{ST}-values ranging from 0.0367 to 0.1374 (**Table 5**).

TABLE 2 | Twelve microsatellite markers developed for *Phyllotreta striolata* in this study.

Locus	Motif	Forward primer	Reverse primer	Size (bp)	FL
PS-03	$(CCG)_9$	AGGTAGGCACTAGATTGGCC	GTGAGCGGCGAAGACAAATG	123	FAM
PS-04	$(AAC)_7$	CAACGCAAAGTTCGCCTGAA	ACCAACACCTTCCCTTCAGTG	124	FAM
PS-06	$(AAG)_7$	AGCAGGATAAGGATTAAGAGTGC	TGGAAGATGCAGTAAGTGTTAACA	138	FAM
PS-07	$(CCG)_7$	TCCAACACCATTAGCGCTCT	GTGAGTGATGCTCCGTCAGT	138	FAM
PS-08	$(ATC)_7$	CGGACGATGAGGACTTTCATC	CCCTCATCCTCTTCTTGTTCTTG	139	HEX
PS-09	$(ATC)_7$	TTTCCAATTTAGCGAGCTCGT	TCGAATGCGATCGTTTAGGAG	140	HEX
PS-13	$(ATC)_7$	AGTGTACCTTGGCTGTCAATAAA	TCTTCATTTCGAATGAGGCCTC	145	HEX
PS-18	$(ATC)_7$	AATTGGTCGTCGACGTCTCC	CACCAAGAGGAGCAACCGG	157	HEX
PS-20	$(ACC)_7$	CGCTCGTCAGTCCGACTTAT	ATGCCGGTGTTGAGCAAGTT	163	ROX
PS-21	$(AAC)_7$	GGTGACCTCTGGCAGAAACA	TGGTTGTTGCGATGTAGCTCT	164	ROX
PS-22	$(CCG)_{10}$	CCCGGTAGTCAACTCGAGTG	CCCAGCGGCCGGTATATTC	168	ROX
PS-33	$(AAC)_8$	AATTGACCGGCAGCGATTTG	GTGGCGGTACAAGCTCAAAC	193	ROX

FL, fluorescent label.

TABLE 3 | Population genetic diversity of *Phyllotreta striolata* collected from southern China.

Population	Mitochondrial DNA							Microsatellite loci						
	N	H	H_d	Pi	S	Tajima's D	Fu's F_S	A_R	P_{AR}	A_T	A_S	Ho	H_E	F_{IS}
AH	24	11	0.717	0.00239	7	−2.10*	−3.05*	4.25	0.08	50	39.49	0.443	0.577	0.236
FJ	24	8	0.594	0.00211	6	−1.65	−1.36	3.83	0.25	43	34.26	0.450	0.533	0.159
GD	24	9	0.565	0.00439	7	−2.26**	−3.68**	4.58	0.42	47	37.66	0.507	0.576	0.125
GX	24	13	0.931	0.00497	8	−0.10	0.57	3.83	0.08	30	30.00	0.389	0.486	0.203
HN	23	9	0.636	0.00260	11	−2.07*	−2.47	4.17	0	44	34.22	0.387	0.512	0.273
JS	24	5	0.493	0.00103	4	−1.52	−1.61	4.58	0.25	48	37.22	0.426	0.560	0.237
JX	22	5	0.338	0.00059	3	−1.88*	−2.86*	4.00	0.08	43	35.83	0.412	0.513	0.201
ZJ	23	4	0.383	0.00118	3	−1.88*	−2.90*	4.42	0.25	48	37.11	0.437	0.555	0.217

*N, number of individuals used in the analysis; H, number of haplotypes; H_d, haplotype diversity; Pi, nucleotide diversity; S, number of polymorphic sites; A_R, allelic richness for 24 individuals per population; P_{AR}, private allelic richness for 24 individuals per population; AT, total number of alleles; As, standardized total number of alleles (for 7 individuals); Ho, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. *Means the difference is significant at the 0.05 level. **Means the difference is significant at the 0.01 level.*

TABLE 4 | Pairwise genetic distance among 34 mitochondrial *cox1* haplotypes.

	hap1	hap2	hap3	hap4	hap5	hap6	hap7	hap8	hap9	hap10	hap11	hap12	hap13	hap14	hap15	hap16	hap17	hap18	hap19	hap20	hap21	hap22	hap23	hap24	hap25	hap26	hap27	hap28	hap29	hap30	hap31	hap32	hap33	hap34
hap1		0.036	0.057	0.062	0.056	0.052	0.061	0.056	0.111	0.057	0.057	0.059	0.057	0.090	0.061	0.061	0.082	0.062	0.086	0.061	0.124	0.116	0.101	0.062	0.061	0.058	0.059	0.083	0.059	0.064	0.081	0.114	0.058	0.059
hap2	0.032		0.035	0.037	0.035	0.036	0.035	0.036	0.086	0.035	0.035	0.035	0.034	0.062	0.037	0.036	0.058	0.037	0.061	0.035	0.087	0.087	0.073	0.037	0.037	0.038	0.034	0.060	0.038	0.087	0.058	0.156	0.037	0.084
hap3	0.069	0.032		0.060	0.056	0.051	0.057	0.060	0.110	0.055	0.056	0.058	0.055	0.090	0.060	0.057	0.080	0.057	0.086	0.055	0.117	0.115	0.096	0.058	0.059	0.058	0.056	0.084	0.059	0.119	0.084	0.202	0.059	0.110
hap4	0.069	0.032	0.069		0.059	0.055	0.059	0.061	0.117	0.058	0.057	0.061	0.059	0.089	0.062	0.060	0.088	0.059	0.087	0.060	0.122	0.121	0.101	0.062	0.061	0.062	0.063	0.084	0.060	0.124	0.082	0.246	0.059	0.119
hap5	0.069	0.032	0.069	0.069		0.051	0.060	0.056	0.116	0.058	0.057	0.059	0.059	0.087	0.059	0.054	0.036	0.056	0.086	0.058	0.118	0.116	0.053	0.059	0.056	0.058	0.054	0.037	0.060	0.115	0.081	0.121	0.058	0.062
hap6	0.066	0.032	0.066	0.066	0.066		0.052	0.055	0.075	0.053	0.054	0.054	0.052	0.076	0.053	0.054	0.073	0.055	0.076	0.051	0.105	0.075	0.089	0.054	0.055	0.058	0.053	0.074	0.054	0.099	0.072	0.178	0.053	0.097
hap7	0.069	0.032	0.069	0.069	0.069	0.066		0.058	0.114	0.055	0.057	0.057	0.056	0.083	0.057	0.059	0.085	0.058	0.083	0.060	0.118	0.114	0.051	0.057	0.060	0.058	0.054	0.090	0.058	0.118	0.083	0.238	0.058	0.121
hap8	0.069	0.032	0.069	0.069	0.069	0.066	0.069		0.122	0.057	0.059	0.056	0.055	0.093	0.061	0.060	0.080	0.059	0.088	0.056	0.116	0.117	0.098	0.063	0.060	0.060	0.057	0.084	0.061	0.113	0.083	0.201	0.058	0.107
hap9	0.160	0.111	0.160	0.160	0.160	0.105	0.160	0.160		0.119	0.120	0.115	0.112	0.159	0.114	0.118	0.165	0.118	0.169	0.114	0.271	0.114	0.191	0.118	0.122	0.126	0.118	0.166	0.125	0.229	0.166	0.657	0.123	0.257
hap10	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160		0.057	0.058	0.059	0.085	0.058	0.058	0.082	0.058	0.089	0.057	0.118	0.122	0.098	0.059	0.059	0.063	0.055	0.083	0.063	0.115	0.083	0.216	0.060	0.116
hap11	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069		0.057	0.056	0.087	0.062	0.057	0.083	0.058	0.090	0.056	0.112	0.120	0.099	0.061	0.059	0.060	0.055	0.082	0.060	0.120	0.081	0.207	0.057	0.115
hap12	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069		0.057	0.086	0.057	0.056	0.084	0.061	0.082	0.058	0.114	0.059	0.100	0.058	0.059	0.060	0.058	0.087	0.058	0.115	0.081	0.212	0.057	0.119
hap13	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069		0.088	0.057	0.055	0.086	0.057	0.082	0.055	0.115	0.119	0.100	0.060	0.059	0.057	0.054	0.088	0.057	0.111	0.081	0.205	0.057	0.114
hap14	0.111	0.069	0.111	0.111	0.111	0.105	0.111	0.111	0.217	0.111	0.111	0.111	0.111		0.086	0.085	0.115	0.088	0.119	0.091	0.174	0.186	0.136	0.036	0.089	0.082	0.086	0.128	0.089	0.184	0.120	0.382	0.089	0.170
hap15	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111		0.058	0.083	0.059	0.038	0.060	0.119	0.120	0.098	0.060	0.058	0.059	0.059	0.087	0.065	0.058	0.086	0.232	0.064	0.126
hap16	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069		0.081	0.057	0.086	0.057	0.119	0.118	0.096	0.058	0.060	0.064	0.056	0.079	0.060	0.120	0.085	0.118	0.060	0.110
hap17	0.111	0.069	0.111	0.111	0.032	0.105	0.111	0.111	0.217	0.111	0.111	0.111	0.111	0.160	0.111	0.111		0.080	0.120	0.084	0.164	0.166	0.075	0.081	0.083	0.082	0.079	0.058	0.083	0.155	0.108	0.172	0.082	0.086
hap18	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111		0.089	0.059	0.124	0.130	0.096	0.059	0.062	0.061	0.058	0.082	0.062	0.118	0.085	0.222	0.060	0.116
hap19	0.111	0.069	0.111	0.111	0.111	0.105	0.111	0.111	0.217	0.111	0.111	0.111	0.111	0.160	0.032	0.111	0.160	0.111		0.089	0.168	0.164	0.131	0.086	0.088	0.088	0.085	0.122	0.095	0.085	0.118	0.346	0.093	0.215
hap20	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111	0.069	0.111		0.124	0.122	0.105	0.063	0.059	0.060	0.055	0.085	0.058	0.122	0.084	0.222	0.058	0.119
hap21	0.160	0.111	0.160	0.160	0.160	0.151	0.160	0.160	0.286	0.160	0.160	0.160	0.160	0.217	0.160	0.160	0.217	0.160	0.217	0.160		0.285	0.137	0.116	0.116	0.119	0.115	0.223	0.121	0.232	0.152	0.245	0.114	0.237
hap22	0.160	0.111	0.160	0.160	0.160	0.105	0.160	0.160	0.160	0.160	0.160	0.069	0.160	0.217	0.160	0.160	0.217	0.160	0.217	0.160	0.286		0.175	0.126	0.118	0.129	0.117	0.166	0.119	0.235	0.146	0.487	0.112	0.242
hap23	0.151	0.105	0.151	0.151	0.066	0.146	0.066	0.151	0.266	0.151	0.151	0.151	0.151	0.204	0.151	0.151	0.105	0.151	0.204	0.151	0.204	0.266		0.096	0.096	0.099	0.094	0.077	0.098	0.186	0.128	0.206	0.098	0.108
hap24	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.032	0.069	0.069	0.111	0.069	0.111	0.069	0.160	0.160	0.151		0.061	0.057	0.058	0.090	0.060	0.123	0.087	0.221	0.060	0.118
hap25	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111	0.069	0.111	0.069	0.160	0.160	0.151	0.069		0.062	0.061	0.079	0.060	0.114	0.085	0.213	0.060	0.113
hap26	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111	0.069	0.111	0.069	0.160	0.160	0.151	0.069	0.069		0.059	0.083	0.062	0.111	0.084	0.225	0.059	0.115
hap27	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111	0.069	0.111	0.069	0.160	0.160	0.151	0.069	0.069	0.069		0.081	0.058	0.117	0.082	0.212	0.057	0.115
hap28	0.111	0.069	0.111	0.111	0.032	0.105	0.111	0.111	0.217	0.111	0.111	0.111	0.111	0.160	0.111	0.111	0.069	0.111	0.160	0.111	0.217	0.217	0.105	0.111	0.111	0.111	0.111		0.086	0.167	0.117	0.167	0.086	0.086
hap29	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111	0.069	0.111	0.069	0.160	0.160	0.151	0.069	0.069	0.069	0.069	0.111		0.128	0.087	0.224	0.063	0.119
hap30	0.069	0.111	0.160	0.160	0.160	0.151	0.160	0.160	0.286	0.160	0.160	0.160	0.160	0.217	0.069	0.160	0.217	0.160	0.111	0.160	0.286	0.286	0.266	0.160	0.160	0.160	0.160	0.217	0.160		0.086	0.228	0.061	0.123
hap31	0.111	0.069	0.111	0.111	0.111	0.105	0.111	0.111	0.217	0.111	0.111	0.111	0.111	0.160	0.111	0.111	0.160	0.111	0.160	0.111	0.217	0.217	0.204	0.111	0.111	0.111	0.111	0.160	0.111	0.111		0.376	0.036	0.154
hap32	0.160	0.217	0.286	0.286	0.160	0.266	0.286	0.286	0.471	0.286	0.286	0.286	0.286	0.368	0.286	0.160	0.217	0.286	0.368	0.286	0.286	0.471	0.266	0.286	0.286	0.286	0.286	0.217	0.286	0.286	0.368		0.227	0.061
hap33	0.069	0.032	0.069	0.069	0.069	0.066	0.069	0.069	0.160	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.111	0.069	0.111	0.069	0.160	0.160	0.151	0.069	0.069	0.069	0.069	0.111	0.069	0.069	0.032	0.286		0.114
hap34	0.069	0.111	0.160	0.160	0.069	0.151	0.160	0.160	0.286	0.160	0.160	0.160	0.160	0.217	0.160	0.160	0.111	0.160	0.217	0.160	0.286	0.286	0.151	0.160	0.160	0.160	0.160	0.111	0.160	0.160	0.217	0.069	0.160	

Data of from the upper triangle refer to the standard error.

TABLE 5 | Pairwise population genetic differentiation (F_{ST}, lower triangle) and *P*-values (upper triangle) among eight populations of *Phyllotreta striolata* based on haplotypes of the *cox1* gene.

Population	AH	FJ	GD	GX	HN	JS	JX	ZJ
AH		0.6937	0.8739	0.0180	0.1171	0.0270	0.0811	0.7478
FJ	–0.0119		0.4775	0.0811	0.1261	0.0090	0.0360	0.6757
GD	–0.0129	–0.0012		0.0360	0.0991	0.0090	0.1081	0.4865
GX	**0.0520**	0.0367	**0.0458**		0.0000	0.0000	0.0000	0.0000
HN	0.0160	0.0171	0.0157	**0.0678**		0.3514	0.1261	0.2523
JS	**0.0462**	**0.0598**	**0.0365**	**0.1105**	0.0038		0.1441	0.0270
JX	0.0487	**0.0577**	0.0302	**0.1374**	0.0162	0.0197		0.1351
ZJ	–0.0187	–0.0062	–0.0062	**0.0807**	0.0126	**0.0433**	0.0405	

F_{ST}-values with significant differences indicated in bold.

TABLE 6 | Pairwise population genetic differentiation (F_{ST}, lower triangle) and *P*-values (upper triangle) among eight populations of *Phyllotreta striolata* based on microsatellites.

Population	AH	FJ	GD	GX	HN	JS	JX	ZJ
AH		0.0155	0.0072	<0.0010	<0.0010	0.3549	0.0227	0.0947
FJ	**0.0092 (0.0071)**		0.0159	0.0103	0.0371	<0.0010	0.7549	0.0021
GD	**0.0147 (0.0135)**	**0.0045 (0.0033)**		0.2558	0.0827	<0.0010	0.0690	0.0550
GX	**0.0392 (0.0340)**	**0.0291 (0.0246)**	0.0203 (0.0187)		0.7473	0.0203	0.0767	0.4836
HN	**0.0298 (0.0301)**	**0.0049 (0.0066)**	0.0003 (0.0034)	0.0032 (0.0027)		0.0091	0.6325	0.3365
JS	0.0081 (0.0092)	**0.0406 (0.0291)**	**0.0419 (0.0341)**	**0.0458 (0.0324)**	**0.0379 (0.0250)**		0.0030	0.1428
JX	**0.0136 (0.01147)**	–0.0125 (–0.0092)	**–0.0011 (–0.0007)**	0.0256 (0.0219)	–0.0042 (–0.0012)	**0.0345 (0.0245)**		0.3211
ZJ	0.0049 (0.0055)	**0.0119 (0.0098)**	0.0038 (0.0041)	0.0091 (0.0069)	0.0006 (0.0029)	0.0057 (0.00413)	0.0048 (0.0049)	

F_{ST}-values were also calculated with excluding of null alleles (data in parentheses). F_{ST}-values with significant difference were in bold.

Based on the 12 developed microsatellite markers, we evaluated the genetic diversity in eight populations of SFB. Significant departures from HWE ($p < 0.05$) were detected in 53 of the 96 population-locus combinations after sequential Bonferroni correction. The population-locus pairs colonized by primer PS-22 all showed significant departures from HWE. Thirty-five of the 528 locus-locus pairs showed linkage disequilibrium in at least one population ($p < 0.05$), whereas five (S08-S21, S20-S13, S09-S22, S22-S33, and S07-S33 pair) of 66 locus pairs showed linkage disequilibrium across all populations.

Genetic diversity parameters varied among populations. The observed (H_o) and expected (H_E) heterozygosity values ranged from 0.387 to 0.507 and from 0.486 to 0.577, respectively. The total number of alleles was the highest in population AH, with a value of 50. In calculating the standardized total number of alleles, we removed locus S06 because of the failure of amplification in the population JX. The inbreeding coefficient of all populations ranged from 0.125 to 0.273 (**Table 3**). F_{ST}-values ranged from –0.0125 (JX-FJ) to 0.0458 (JS-GX). F_{ST}-values of populations pairs JX-FJ, JX-GD, and JX-GX are below 0 (**Table 6**). F_{ST}-values had significant differences among 15 populations (AH-FJ, AH-GD, AH-GX, AH-HN, AH-JX, FJ-GD, FJ-GX, FJ-HN, FJ-JS, FJ-ZJ, GD-JS, GD-JX, GX-JS, HN-JS, and JS-JX). The G_{ST} of total alleles is 0.011 with a N_m of 44.88, indicating high geneflow among SFB populations. We also calculated the F_{ST}-values with excluding of ENA but the results are similar to that without ENA (**Table 6**). Populations GX and JS have the highest geographical distance and genetic distance. However, the JX and FJ have the nearest geographical distance but not the least genetic distance. The Principal Coordinate Analysis (PCoA) showed no obvious geographic structure (**Figure 2**). The first and second principal coordinates explained 21.78 and 16.65% of the total variation, respectively.

To determine whether the genetic distance in SFB is significantly associated with geographic distance, we conducted Mantel tests between pairwise genetic differentiation $[F_{ST}/(1 - F_{ST})]$ and geographical distance matrix based on haplotypes and microsatellites (**Figure 3**). The relationships of genetic distance and geographic distance were not identical based on microsatellites ($R^2 = 0.2373$, $P = 0.014$) and mtDNA ($R^2 = 0.0365$, $P = 0.155$).

Population Genetic Structure and Phylogenetic Relationship Analysis

We reconstructed the phylogenetic tree based on thirty-two haplotypes (**Figure 4**). Haplotypes from different geographical regions were mixed in the phylogenetic tree, which was consistent with PCoA analysis. We did not find any geographically related clades with low supporting data, except for one clade composed of two haplotypes (30 and 32) unique to populations outside of China, and another clade composed of three haplotypes (28, 29, and 31) unique to populations outside of China.

The STRUCTURE analysis indicated that the optimal *K*-value was two. There was a lack of population structure when K increased from 2 to 3 (**Figure 5A**). DAPC analysis revealed similar patterns to those obtained from the

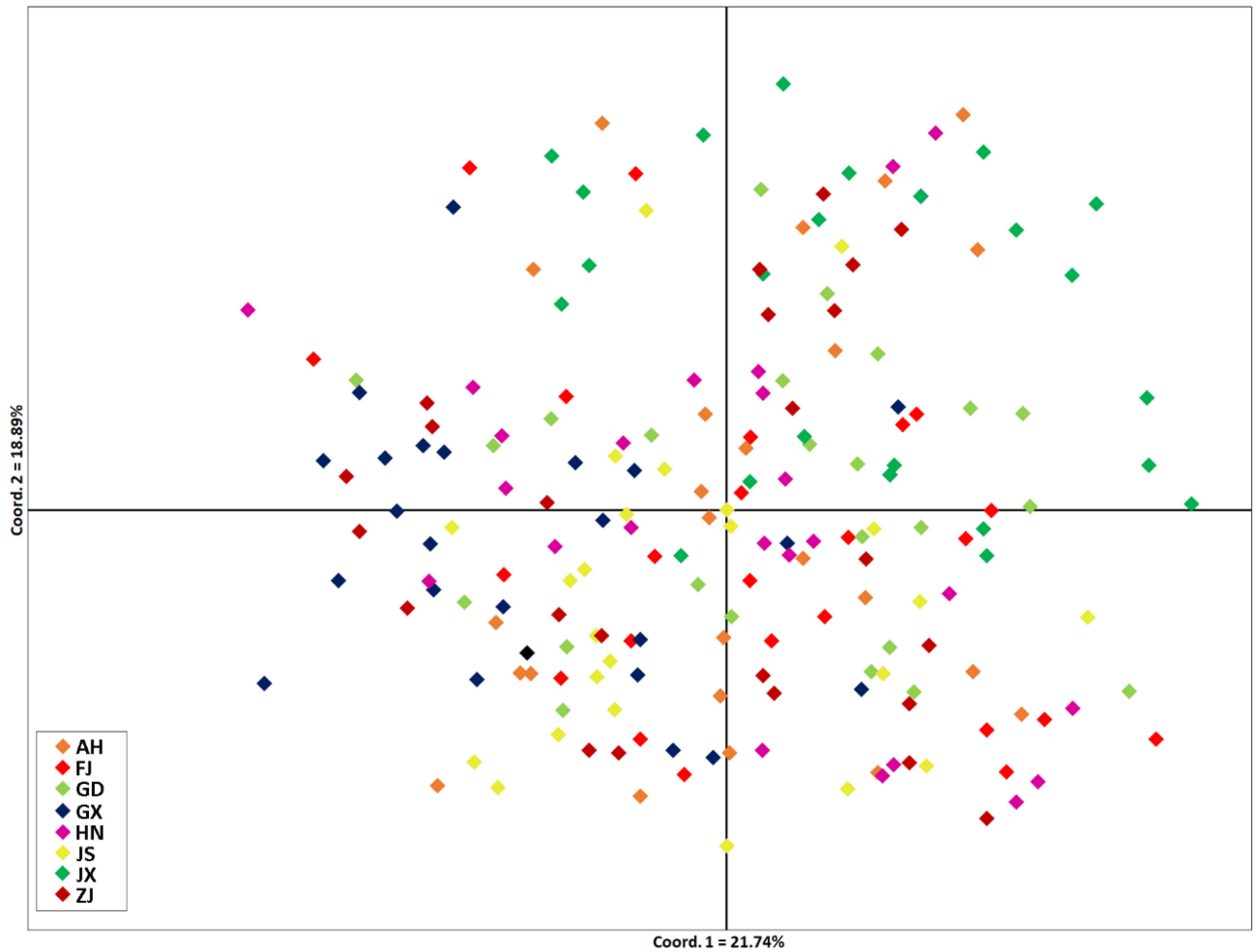

FIGURE 2 | PCoA based on pairwise Nei's genetic distance estimates for the populations.

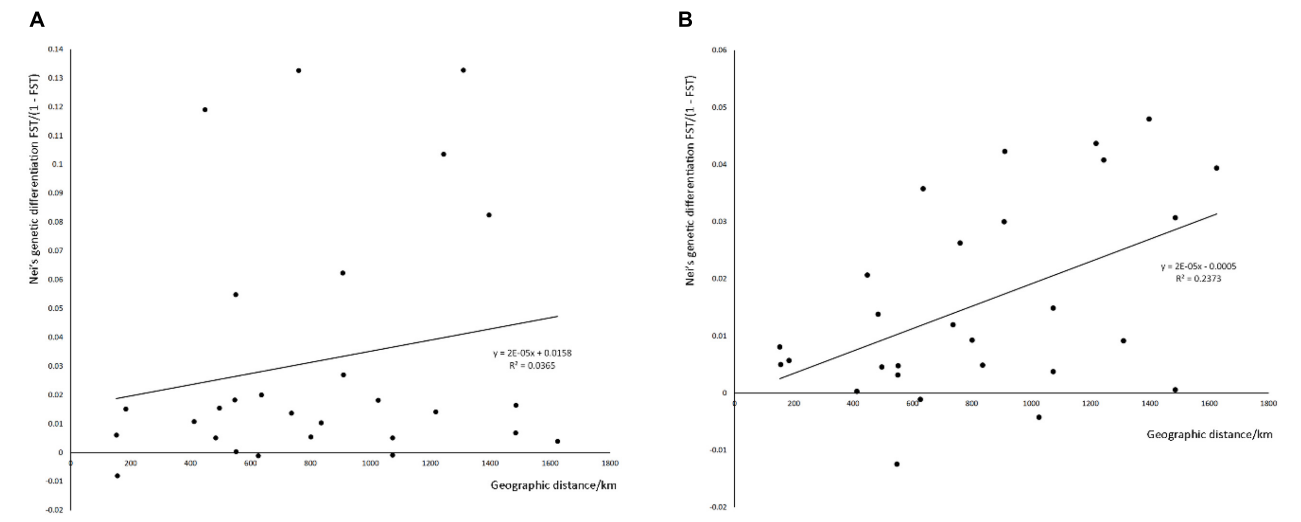

FIGURE 3 | Mantel test of the geographic distance and Nei's genetic distance based on haplotypes of *cox1* gene **(A)** and microsatellites **(B)**.

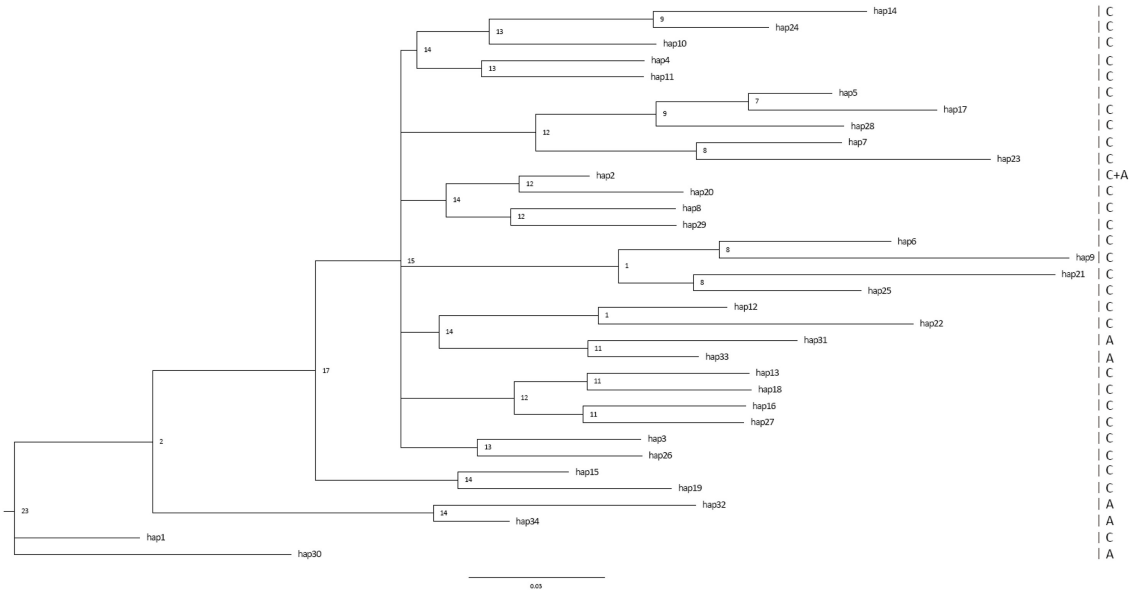

FIGURE 4 | Bayesian-inference phylogenetic relationships among mitochondrial *cox1* haplotypes of *Phyllotreta striolata* worldwide. C, haplotypes present in China. A, haplotypes present outside China.

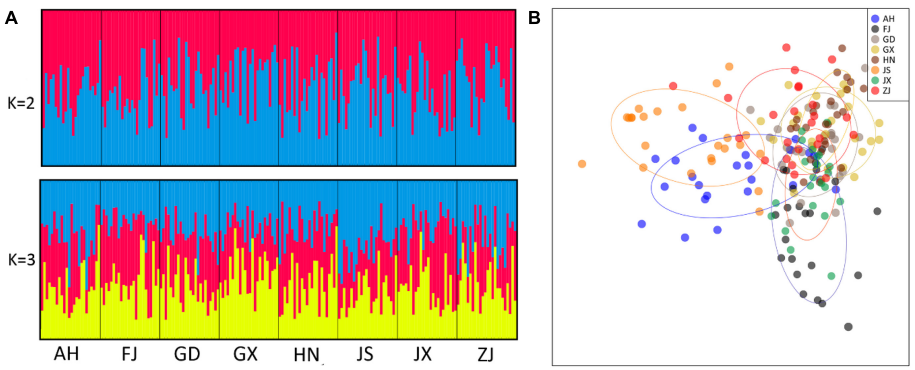

FIGURE 5 | Genetic structure of *Phyllotreta striolata* populations from southern China inferred from STRUCTURE **(A)** and DAPC **(B)** analyses based on 12 microsatellite loci. The optimal number of clusters for the STRUCTURE analysis was three; *K* = 2 and 3 are presented **(A)** arranged by population. DAPC analysis shows all populations are clumping together **(B)**.

STRUCTURE clustering analyses with all populations clumping together (**Figure 5B**).

DISCUSSION

This study developed universal microsatellite markers of SFB based on a *de novo* assembled draft genome. Both mitochondrial DNA and microsatellite markers point to the low population genetic diversity of SFB in southern China. The haplotype analyses using partial mitochondrial *cox1* genes also confirms the low genetic diversity in Europe and Asia since only two haplotypes were identified from the public databases. A lack of population structure was supported by population genetic structure analysis results using STRUCTURE and DAPC.

The mononucleotide repeat sites included four types as $(A)_n$, $(T)_n$, $(C)_n$, and $(G)_n$. The number of $(A)_n$ and $(T)_n$ far exceeded that of $(C)_n$ and $(G)_n$ in SFB, which is similar to other eukaryotic species (Katti et al., 2001). $(A)_n$, $(AT)_n$, and $(AAT)_n$ were the most frequent motifs in SFB, as in other beetle species (Song et al., 2020). The frequency and density of microsatellite markers varied among species (Selkoe and Toonen, 2010; Xu et al., 2017; Liu et al., 2019). The dominant repeats in SFB are dinucleotides except for an occasional mononucleotide. Interestingly, this differs from other beetle species that have been sequenced in the Family Chrysomelidae, in which the dominant repeats are trinucleotide or tetranucleotide repeats in *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae) and *Diabrotica virgifera* (Coleoptera, Chrysomelidae), respectively (Kim et al., 2008; Liu et al., 2018).

In general, the results based on microsatellites and haplotypes of the *cox1* gene support the notion that the sampled populations of SFB have low genetic variation and lack population genetic structure in southern China, as suggested by multiple lines of analyses such as pairwise F-statistics, PCoA, DAPC, STRUCTURE and phylogenetic reconstruction. The lack of population genetic structure was found in some species, such as recently introduced species *Obolodiplosis robiniae* and *Frankliniella occidentalis* (Shang et al., 2015; Cao et al., 2017) or migratory species like *Plutella xylostella*, whose Sichuan populations are sources populations for northern immigrants, and southern China and Yunnan populations are sources populations for central-eastern populations (Chen et al., 2020). In order to escape suboptimal environmental or weather conditions, some insects have annual migrations (Zhan et al., 2011). In Coleoptera, some species, like *Leptinotarsa decemlineata* and *Pachysternum capense* can migrate (Ferro et al., 1991; Greń and Górz, 2020). We did not find a records of migration events for the of SFB and studied have shown that it has been established in Eurasia for several centuries (Rousseau and Lesage, 2016). Our hypothesis, that the low levels of genetic diversity and population differentiation for the SLB are due to high levels of gene flow among populations, needs to be further tested.

Consistently low variation among groups is indicative of high levels of gene flow across the geographic range as seen microsatellite data. Pairwise comparisons of F_{ST}-values from mtDNA data are also consistent with high gene flow across the range, with generally low F_{ST}-values between populations. Haplotypes (n = 2) widely exist in all populations also suggest a high-level of gene flow among 8 populations. Cruciferous vegetables are a kind of common vegetable in China and is the preferential host of SFB. The high gene flow maybe due to the transportation of fresh vegetable from south to north in China, such as from Anhui Province to Zhejiang Province. The passive transportation accelerates gene flow among different populations (Martinez-Hernandez et al., 2021). The high geneflow, however, may be the evidence of its migration (Seymour et al., 2016; Yang et al., 2020).

But there are also some divergences between these two types of molecular markers. A moderate genetic differentiation was observed between GX and other populations based on genetic distances of mtDNA, which were not observed based on microsatellites. Because mtDNA is sensitive to founder effects and small population size, the probable loss or gain of a mtDNA haplotype will be greater for small populations (Roderick, 1996). Furthermore, the Mantel test of microsatellites suggests evidence of a positive relationship in genetic distances (F_{ST}) between genetic and geographic distances, while the result based on mtDNA had no significant difference. This may be caused by the use of only a single marker of mtDNA vs. 12 markers of microsatellite. Different parts of mtDNA evolve at different rates, thus different parts of mitochondrial DNA should be considered for future studies to find higher-level population differentiation (Avise et al., 1992). The significant Mantel test suggests that while genetic differences between populations are rather small, they tend to accumulate as geographic distances increase, supporting the non-negligible population structure.

We found that the population collected from Guangxi province of southwest China had the highest number of unique haplotypes. Many species in East Asia were found to have originated from southwestern China and expanded their distribution range northward during the interglacial periods in the Quaternary (Dong et al., 2013; Wei et al., 2015; Yang et al., 2020). Our study did not include samples collected from southwestern areas such as Yunnan and Sichuan. The inclusion of Guangxi populations and the relatively higher level of genetic diversity in this population may indicate that the SFB may also have originated from southwestern China and subsequently dispersed to other areas.

CONCLUSION

In conclusion, our study provides a draft genome and a set of microsatellites developed from the genome. Based on these microsatellites and a segment of mitochondrial gene, we investigated the population genetic structure of the SFB in southern China, where it is a common pest. We found a lack of population differentiation of SFB among populations in southern China, which might be caused by high frequencies of gene flow among populations. Either passive transportation or migration events will accelerate the gene flow among different populations. Species collected from Guangxi Province showed more genetic divergence than others based on mitochondrial gene which indicates that the SFB may originate from southwestern China. In consideration of the limited collection range, the hypothesized movements need further investigation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, PRJNA761897. Data are also available on the Barcode of Life Datasystems (BOLD) database under Project–SFB "Barcoding of *Phyllotreta striolata* in southern China" (doi: 10.5883/DS-188PSLQ).

AUTHOR CONTRIBUTIONS

QL analyzed the data and wrote the article. QL and G-ML collected the data. Y-LZ and S-JW revised the article. All authors contributed significantly to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.775414/full#supplementary-material

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