Zoological Systematics, 44(2): 100–110 (April 2019), DOI: 10.11865/zs.201905

ORIGINAL ARTICLE

Genome-wide developed microsatellite markers for the melon thrips *Thrips palmi* Karny (Thysanoptera: Thripidae)

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Abstract The melon thrips, *Thrips palmi* Karny, is an economically important pest on many vegetables from Solanaceae and Cucurbitaceae. In this study, we developed novel microsatellite markers for *Thrips palmi.* First, we obtained randomly sequenced regions from the genome using next-generation sequencing and assembled 356 Mb genomic sequences. In total, 155,789 microsatellites were identified from the genomic sequences, of which, 64.02% were loci of dinucleotide repeat. Sixty primer pairs were initially validated in seven individuals. Thirty-five polymorphic markers were retained according to the amplification efficiency and polymorphism. These 35 microsatellite loci were then assessed in 96 individuals in four geographical populations collected from China. The allele numbers ranged from 2 to 19 with an average value of 6.9 per locus, while the polymorphism information content ranged from 0.2219 to 0.8490. The observed and expected heterozygosity varied from 0.5252 to 0.5367 and 0.5934 to 0.6148, respectively. Genetic structure was found between populations from southern and northern China. These novel microsatellite markers developed in our study provide abundant molecular markers for the genetic study of *T. palmi*.

Key words Microsatellite, genetic marker, genome, population genetic structure.

1 Introduction

The melon thrips, *Thrips palmi* Karny (Thysanoptera, Thripidae), is an important pest of vegetables from Cucurbitaceae and Solanaceae (Cannon *et al.*, 2007). It causes damage to vegetables through feeding, laying eggs or transmitting viruses (Reitz, 2009; Reitz *et al.*, 2011a, b). *T. palmi* was first described in 1925 from specimens collected from tobacco in Sumatra in Indonesia, and has spread widely in tropical and subtropical regions (Walker, 1994). In China, *T. palmi* was first recorded in Taiwan in 1979 (Chang, 1991). It was dispersed to Hubei, Zhejiang, Jiangsu and Shanghai of China in the 1990s and to Beijing in 2001 (Yi & Liang, 2001). *T. palmi* is presently a major pest in greenhouses on vegetables of Cucurbitaceae and Solanaceae. Due to its heavy damage and potential invasiveness, *T. palmi* has become a species of major quarantine concern, often intercepted on plant material in international trade (Strassen, 1989).

Population genetics analyses can help to identify the capacity of pests to expand their range and track the history of invasion (Lee, 2002; Liu *et al.*, 2016). Based on mitochondrial DNA, population genetic structure of *T. palmi* was found in 21 populations collected from Karnataka, India (Rebijith *et al.*, 2007). Source and invasion history of *T. palmi* in China and other areas remains unclear although there are many historical records on the occurrence of this species. Population genetics study of *T. palmi* many provide complemental information on the demographic history of this species.

An informative set of genetic markers is essential for population genetics studies. The microsatellite is a kind of special

Received 28 January 2019, accepted 20 April 2019
Executive editor: Fuqiang Chen

sequence comprised by tandem repeats of one to six nucleotides. Microsatellite markers are high polymorphic, widely dispersed in both coding and noncoding regions of all prokaryotic and eukaryotic genomes (Rebijith *et al.*, 2007). Due to their codominant inheritance, high polymorphism, easy detection by polymerase chain reaction (PCR), and broad distribution in the genome, microsatellites are widely used for population genetics studies (Bruford & Wayne, 1993; Cao *et al*., 2015, 2016a). However, development of microsatellite markers is usually challenging due to the complicated procedures by using traditional method (Cao *et al.*, 2015) and lack of genomic sequences by using genomic scan method (Wang *et al.*, 2016). Development of next-generation sequencing makes de novo sequencing of genomes in non-model species feasible and consequently facilitated the development of high quality microsatellite markers (Cao *et al.*, 2016b; Ma *et al.*, 2019).

In this study, we developed microsatellites markers for *T. palmi* from the de novo sequenced genomic sequences and validated them in four natural populations from China. These microsatellites can provide insights into genetic diversity, genetic structure and invasion history of this pest species.

2 Materials and Methods

2.1 Sample collection and DNA extraction

A male adult of the *T. palmi* collected from Shouguang, Shandong Province, China was sampled for genomic sequencing. Seven individuals from seven different locations were used for the initial testing of the primer pairs. Additionally, a total of 96 adults were collected from four geographic regions in China, of which 24 samples were from Changsha of the Hunan Province, named HNCS (113°10'37.19"E, 28°15'31.19"N), Shouguang of the Shandong Province, named SDSG (118°33'14.19"E, 36°49'11.09"N), Daxing of the Beijing, named BJDX (116°20'9.79"E, 39°31'0.78"N) and Anshan of Liaoning Province, named LNAS (122°36'27.62"E,41°4'31.80"N). All specimens were stored in absolute ethanol and frozen at -80°C until DNA extraction.

2.2 Sequencing of random genomic sequences

We de novo sequenced a draft genome of *T. palmi* for microsatellite development. A library with a 500-bp insert size was constructed using the Illumina TruSeq DNA PCR-Free HT Library Prep Kit (Illumina, San Diego, CA, USA); the library was sequenced on Illumina NovaSeq platform (Illumina, San Diego, CA, USA) to generate raw reads. The quality of sequences was evaluated by FastQC v 0.11.5 before assembly (Xiao *et al.*, 2015). Low-quality reads were filtered by Trimmomatic 0.36 (Bolger *et al.*, 2014). The genome was assembled using IDBA_UD with kmers from 20 to 140 (Peng *et al.*, 2010). The assembled contigs were used for discovery of microsatellite loci.

2.3 Characterization of microsatellites in the *Thrips palmi* and primer design

We investigated the distribution of microsatellites from the assembled genome by MSDB software (http://msdb.biosv.com/) with a minimum of 12, 7, 5, 5, 5 and five repeats to identify the mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide and hexanucleotide motifs, respectively as previously used (Ma *et al.*, 2019). The average length of microsatellites, the length, total counts, frequency (loci/Mb) and density (loci/bp) of the motif were analyzed (Du *et al.*, 2013).

We used the QDD program to design primers for the identified microsatellite loci (Meglecz *et al.*, 2010). The criteria for searching for primers were as follows according to previous studies (Cao *et al.*, 2016b; Wang *et al.*, 2016; Song *et al.*, 2017). (i) Tri-, tetra-, penta- and hexa-nucleotide motifs were considered with at least more than 6, 6, 5, 5 repeats, respectively; (ii) the size of PCR product ranged from 100 to 350 bp, and 20 bp was considerded as an optimal primer length; (iii) the annealing temperature (Tm) needed to be between 58.0°C and 63.0°C, and the difference in Tm between pairwise primers had to be lower than 5°C. The best locus for each sequence and design strategy of ‘A’ was retained following stringent criteria (Cao *et al.*, 2016b). The minimum distance between the 3’ end of two primer pairs and their target region had to be > 10 bp.

2.4 Primer validation and genotyping

Sixty primer pairs from different contigs were used for initial testing on seven individuals randomly selected from the four populations. Primer pairs generating polymorphic loci and successfully amplified in at least five individuals were

retained for population-level genotyping in four populations from China. Polymorphic loci with PCR amplification rate higher than 75% were maintained for genotyping.

In the genotyping process, we added a universal primer tail C (PC tail) (5'-CAGGACCAGGCTA CCGTG-3') to the 5' end of the forward primer (Blacket *et al.*, 2012). Conditions for PCR amplification were described in previous publications (Cao *et al.*, 2016b; Song *et al.*, 2017). Briefly, PCR amplification was performed in 10 μL volume reactions using a universal primer with PC tail, a PC-tail modified forward primer and a reserve primer. Randomly selected PCR products were visualized on agarose gel (1.0%) electrophoresis. PCR products were analyzed using ABI 3730xl DNA Analyzer (Applied Biosystems, Foster, CA, USA) with the GeneScan 500 LIZ size standard (Applied Biosystems) by Tsingke Biotechnology Co. Ltd (Beijing, China). Microsatellite loci were genotyped using GENEMAPPER 4.0 (Applied Biosystems, USA) and checked for stuttering and large allele dropout using MICRO-CHECKER version 2.2.3 (van Oosterhout *et al.*, 2004).

2.5 Genetic diversity analysis

Hardy-Weinberg equilibrium (HWE) for each locus at each population, linkage disequilibrium between each pair of loci at each population, pairwise mean population differentiation (FST) and inbreeding coefficients (FIS) were estimated by GENEPOP version 4.2.1 (Rousset, 2008). The number of alleles, observed heterozygosity (HO), expected heterozygosity (HE) and polymorphism information content (PIC) were calculated by the macros Microsatellite Tools. Putative loci under selection were detected with two options: "neutral mean FST" and "force mean FST" by LOSITAN (Antao *et al.*, 2008).

2.6 Population genetic structure analysis

STRUCTURE version 2.3.4 (Pritchard *et al.*, 2000) was used for population genetic structure analysis. An admixture model with correlated allele frequencies was chosen. Thirty replicates for each K (from 1 to 10) were run with 200,000 Markov chain Monte Carlo (MCMC) iterations after a burn-in of 100,000 iterations. The optimal value of K was determined using the Delta (K) method (Evanno *et al.*, 2005) by submitting the outputs of STRUCTURE to STRUCTURE HARVESTER WEB version 0.6.94 (Earl & Vonholdt, 2012). The membership coefficient matrices (Q-matrices) of replicated runs for each K were combined using CLUMPP version 1.1.2 (Jakobsson & Rosenberg, 2007) with the Greedy algorithm, and then visualized using DISTRUCT version 1.1 (Rosenberg, 2004). To identify the number of different genetic clusters, discriminant analysis of principal components (DAPC) was performed using adegenet version 2.0.1 (Jombart, 2008) in the R environment.

3 Results and Discussion

3.1 Genome sequencing and assembly

We obtained 187,807,448 paired-end (PE) raw reads in total of 28.17 Gb with read length of 150 base pairs (bp) from DNA library. After removing low-quality reads, the remaining high-quality reads were assembled into 777,895 scaffolds. The length of total scaffolds was 356 Mb, with mean size of 569 bp and N50 of 1123 bp. For the first time, we sequenced random genomic sequences of *T. palmi*. The genome size is comparable to that of other thysanopteran species of *Frankliniella occidentalis* (380 Mb, https://www.hgsc.bcm.edu/arthropods/western-flower-thrips-genome-project). The assembled sequences could be used as references for genome-wide survey of microsatellites. Microsatellites can be developed from coding regions of EST sequences (Yang *et al.*, 2012; Li *et al.*, 2015) and transcriptomes (Luo *et al.*, 2014). Microsatellite loci developed on a genome-wide scale can be located at coding and non-coding regions. These developed microsatellite loci can be further selected with stringent criteria to improve population genetics analysis (Cao *et al.*, 2017).

3.2 Characterization of microsatellites in *Thrips palmi*

A total of 155,789 microsatellite loci were discovered from the assembled scaffolds. Averagely, there is one microsatellite locus per 2285 bp in the genome of *T. palmi*. The abundance of microsatellite loci in *T. palmi* is similar to insects, such as *F. occidentalis* (one locus per 2873 bp) (Cao *et al.*, 2016b) and *Phenacoccus solenopsis* (one locus per 2309 bp) (Ma *et al.*, 2019), but higher than lepidopteran species (Wang *et al.*, 2016; Song *et al.*, 2017). The detected microsatellites included 99,732 (64.02%) dinucleotide, 20,887 (13.41%) trinucleotide, 22,252 (14.28%) tetranucleotide, 12,537 (8.05%) pentanucleotide and 381 (0.24%) hexanucleotide repeats. Dinucleotide repeats are more frequent than the higher order motif

as in most insect species (Rebijith *et al.*, 2007; Zhou *et al.*, 2010).

3.3 Development of microsatellite loci for *Thrips palmi*

In total 104,229 microsatellite loci were suitable for primer design. We retained one primer pair for each locus in primer design. Sixty primer pairs designed for loci of three to five nucleotides repeats were selected for initial validation in seven individuals. We discarded loci which resulted in an amplification rate lower than 86% and have no polymorphism. Among the 60 tested loci, 35 loci were polymorphic (Table 1), four loci were monomorphic, 12 loci did not produce any visible amplicon and amplification efficiency of 9 primer pairs is low.

3.4 Genetic diversity of *Thrips palmi* populations

The 35 polymorphic loci were assessed with four *T. palmi* natural populations, including 24 individuals per population. Allele numbers ranged from 2 to 19 with an average value of 6.9 per locus. The observed (HO) and expected (HE) heterozygosity ranged from 0.5252 to 0.5367 and 0.5934 to 0.6148, respectively. The polymorphism information content (PIC) ranged from 0.2219 to 0.8490 (Table 2).

Nine loci in HNCS, 7 loci in SGSG, 7 loci in BJDX and 6 loci in LNAS population significantly deviated from Hardy-Weinberg equilibrium (HWE), while 4 loci (TP4-33, TP3-38, TP4-41 and TP3-49) showed significant value in each population (Table 2), which might be caused by heterozygote deficiencies and the presence of null alleles. The loci TP3-S01 and TP5-S10, TP3-49 and TP3-56 showed significant linkage disequilibrium (corrected by Holm's correction, $p < 0.05$). It is speculated that the linkage disequilibrium observed at certain loci in some populations may be due to substructure of population or bottleneck. The neutrality test showed that TP4-S33 and TP3-S49 were a candidate for positive selection and TP3-S01, TP5-S10 and TP4-S14 for balancing selection. The other 30 of the markers showed no selection pressure (Fig. 1). TP4-S33 and TP3-S49 as well as another ten loci displayed significant deviation from HWE ($P < 0.05$). TP3-S01 and TP5-S10 showed a significant linkage disequilibrium. Null allele frequencies of the 35 microsatellite loci among four populations was low for most loci (Table 3).

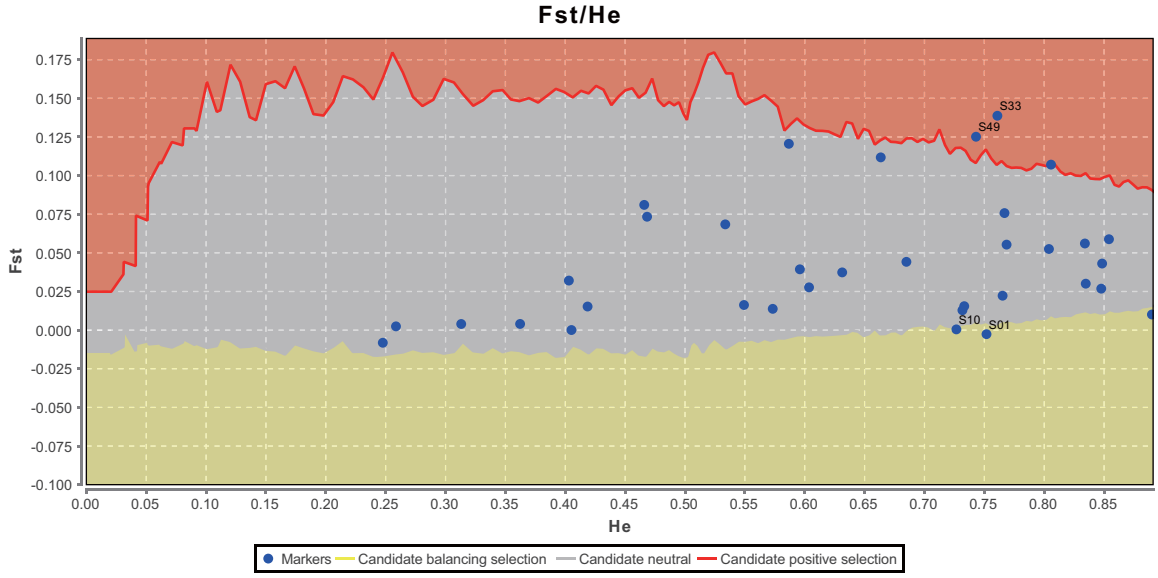

Figure 1. Neutrality tests of the 35 microsatellite loci developed in this study based on four populations of *Thrips palmi* from China. Loci located in the red region are candidates for positive selection, the grey region for neutral, and the yellow region for balancing selection.

3.5 Population genetic structure of *Thrips palmi* based on validated loci

Among geographical populations, FST values ranged from 0.0167 to 0.0867. Value from HNCS and BJDX were higher than those from other population pairs (Table 4). The STRUCTURE analysis of four geographic populations showed the best number of clusters was two. The three northern populations of BJDX, SDSG and LNAS was mainly composed of one cluster, while the southern population HNCS was mainly composed of another cluster (Fig. 2). DAPC analysis showed that

the southern population HNCS and northern population LANS were distantly related to two other populations and all individuals could be clearly divided into different clusters corresponding to their populations (Fig. 3).

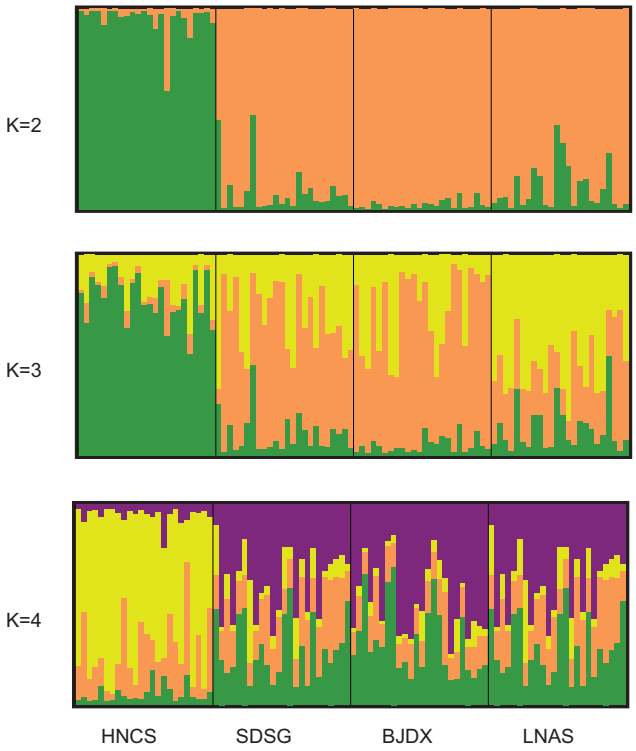

Figure 2. Genetic structures of four *Thrips palmi* populations based on 35 microsatellite markers using the software STRUCTURE. Each bin indicates an individual. Different colors show the identified clusters. The best number of clusters (K) is 2. We show the clustering results when K is 2, 3 and 4. HNCS—Changsha of the Hunan Province; SDSG—Shouguang of Shandong Province; BJDX—Daxing of the Beijing; LNAS—Anshan of Liaoning Province.

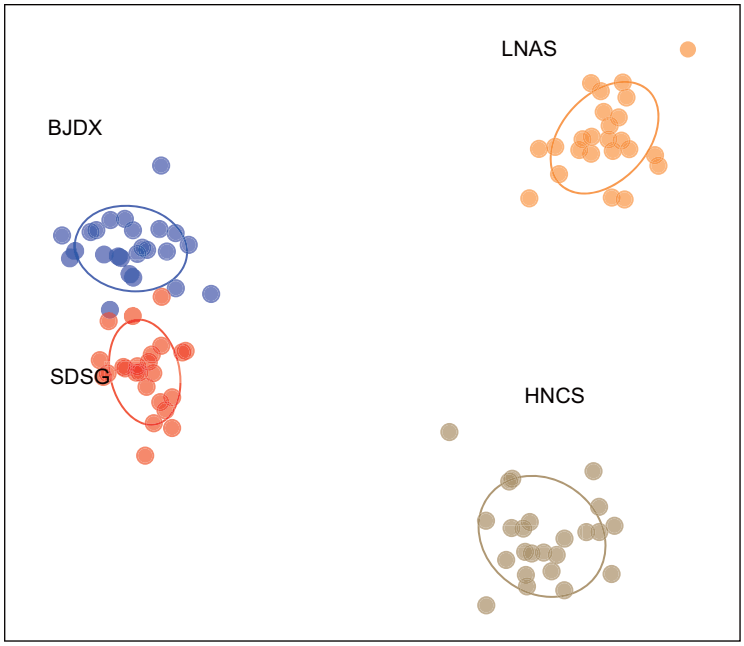

Figure 3. Genetic structures of four *Thrips palmi* populations based on 35 microsatellite markers inferred by DAPC. All individuals were clustered into four clusters coincident with the four populations. Code for the four populations was described in Figure 2.

Table 1. Thirty-five microsatellite markers developed for *Thrips palmi*.

Locus	Dye*	Repeat motif	Allele number	Size range (bp)	PCR product size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
TP3-S01	HEX	$(AAC)_{10}$	3	113–158	106	ACTAAAGAGTGAGCGTCCGC	ACACTCTGGCCCAATCGAAG
TP4-S02	HEX	$(ACAG)_{7}$	5	111–127	112	CCGTCCGTGTCGTATCTGAC	CCTGTCCGTCTGTCAGTGTC
TP5-S03	HEX	$(ACGGC)_{8}$	8	115–167	113	CGCTCCTCATTCGCTCGTAT	TCGACTCGCCATTCATAGGC
TP4-S04	HEX	$(ACAG)_{8}$	5	136–154	126	CAAGACCACGTTTGCCACTG	CCAATTGCCTCGCTGTTACG
TP4-S06	HEX	$(ACAG)_{7}$	12	154–212	139	AAGGAGGCGAATCAAGCGAT	CTCGTCTCGACAAACGGTGA
TP4-S09	HEX	$(ACAG)_{7}$	5	153–173	155	GCGATTTGCGTCCGATTTCA	AGCCAATACCTCTCGTGCTG
TP5-S10	HEX	$(AGCCG)_{6}$	10	169–225	162	CGGTCCCGGAATCACTTAGG	GCCGAATCTATGTCGAGCCA
TP4-S11	HEX	$(AGCC)_{12}$	12	160–256	175	TGTGGGTCTCAGGTAGCAGA	TACGCAGAGTGGAACGGAAC
TP4-S12	HEX	$(ACAG)_{15}$	5	157–198	177	GGTTCACTAATCCTCCGCGT	CCGCCATTAGTGACTCTCCC
TP4-S14	HEX	$(AATG)_{26}$	19	135–217	185	AGTGTCGTGACTCGTTCGTC	ATTCGATTCAACGCGCACTG
TP3-S17	HEX	$(ACC)_{7}$	8	207–271	201	GTCACGTCCATCTACCACCG	CCTGCTGGAGACAACCACTT
TP3-S19	HEX	$(AGG)_{8}$	6	221–237	203	TCGGGATTCGGACTGGTTTG	GACCTTGGTTGGCCTTGACT
TP3-S22	FAM	$(AAC)_{8}$	6	239–258	225	GTAGAGCAGTGCATCGAGCT	ATCTGGGCACTCATTGGACG
TP3-S23	FAM	$(AAC)_{12}$	11	224–259	226	GAAAGCCACGAGGAGATCCC	TTGACAGCGAAGTTTCCCGA
TP4-S24	FAM	$(ACAG)_{19}$	18	192–278	227	CGCGTGCCAGATCAATGAAG	ATCGTCTCTGGACCGATTGC
TP4-S25	FAM	$(ACAG)_{17}$	7	197–245	228	TGCATAACTCTGGGAGGCAC	TGATCCCTTGTCTCGGCCTA
TP4-S27	FAM	$(AGAT)_{22}$	11	218–266	236	TATCGCGCTCTATGCTTCCC	CAGGGAGCTTGATGCAAGGA
TP4-S28	FAM	$(ACGG)_{8}$	5	259–276	241	AAAGCCGAAGCCTCCATACC	CCGACCAACACAGCAACAAG
TP4-S29	FAM	$(AGGC)_{11}$	7	231–259	242	GAGGCTCATTGCACGCAAAT	CCGGAGCGTGAGGAATTGTA
TP4-S30	FAM	$(AGGC)_{10}$	13	250–347	245	CGGACGGACTCGCAATATGA	TTGTGGTCTTCTCCTTGGCC
TP3-S31	FAM	$(AGC)_{9}$	11	254–324	247	GGCTCGTACTGTCTCACCAC	TCGCCGCTTCTTAGAACTCC
TP4-S32	FAM	$(ACAG)_{12}$	9	233–274	249	TGGTCAAGCCAAGCGAATCT	GGCAATCACCAGGGAGGATC
TP4-S33	FAM	$(ACAG)_{9}$	10	200–281	250	AATTACGGAGGTCCAGCACG	GCCATGACTGTCGCTACACT
TP3-S36	FAM	$(ACC)_{7}$	2	278–281	260	GCCCTCTTCACTTCAGCACT	CCACCACACCGGAATATCCC
TP3-S38	ROX	$(AGC)_{7}$	3	274–283	263	CACTCACTCTGGGTGCTGTT	TACACGTCCTTCCACAACGG
TP4-S39	ROX	$(AGGC)_{10}$	4	279–387	265	ACCACTTACGAGGCACAGTG	GAGTCCAGCCGTCGGATTAC

Table 1 (continued)

Locus	Dye*	Repeat motif	Allele number	Size range (bp)	PCR product size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
TP4-S40	ROX	$(ACAG)_{11}$	9	257–305	269	CAAGGTGTGAAGGAAGCCGA	CGGGCTTCGATGAGATCCTC
TP4-S41	ROX	$(AGCC)_{34}$	7	197–289	271	CGGAATCAGTTCGTCGTGGA	TCTACAGCAGCTCGTTTGCA
TP4-S42	ROX	$(ACTC)_{7}$	2	279–291	271	GCGATTGCCTCCGAGTTAGT	CCATGAACGTGTTGGCAGTG
TP3-S49	ROX	$(AAC)_{14}$	8	277–325	299	CAACACTGCACCGGAAGTTG	ATTGGCCGTCGAAAGTCTGT
TP3-S51	ROX	$(ACG)_{7}$	3	315–327	305	GGAGCCCTTGGAGCGAATTA	GGAGCTATCTCGACGTTCCG
TP3-S56	ROX	$(ATC)_{7}$	3	348–354	332	GGGATTTGACGGCAGAGCTA	GGTGGAGCTCGTTCAGAACA
TP4-S57	ROX	$(ATCC)_{9}$	14	320–409	335	AGTGTAGGCGCTCTTTGCTT	GTGACTCTGTGACACGGCTT
TP4-S58	ROX	$(AAAG)_{23}$	16	320–457	341	ACCGCCGTCTCTTGATAAGC	TCACCAGACGGATGCTTACG
TP3-S60	ROX	$(AGC)_{7}$	6	348–367	343	GCATTTCCTCACATCCGCAC	TCTGAACGGGACAACATCGG

*Dye—three fluorescent labels used in PC tail primer for PCR amplification of each locus.

Table 2. Genetic diversity of the four *Thrips palmi* populations calculated using 35 microsatellites markers.*

Locus	Ho				He				P-HWE				F_{IS}				PIC
	HNCS	SDSG	BJDX	LNAS	HNCS	SDSG	BJDX	LNAS	HNCS	SDSG	BJDX	LNAS	HNCS	SDSG	BJDX	LNAS	
TP3-S01	0.792	0.667	0.583	0.667	0.781	0.760	0.746	0.728	0.331	0.248	0.173	0.244	-0.014	0.125	0.222	0.086	0.695
TP4-S02	0.542	0.208	0.417	0.500	0.543	0.311	0.383	0.383	0.537	0.151	1.000	0.272	0.003	0.335	-0.090	-0.314	0.335
TP5-S03	0.435	0.625	0.375	0.583	0.557	0.710	0.653	0.699	0.201	0.626	0.035	0.146	0.223	0.122	0.431	0.168	0.592
TP4-S04	0.500	0.542	0.417	0.542	0.520	0.616	0.533	0.688	0.383	0.243	0.039	0.299	0.040	0.123	0.222	0.216	0.530
TP4-S06	0.583	0.708	0.542	0.708	0.694	0.744	0.698	0.754	0.036	0.110	0.233	0.273	0.163	0.049	0.227	0.061	0.666
TP4-S09	0.333	0.375	0.417	0.333	0.415	0.365	0.364	0.299	0.049	0.693	1.000	1.000	0.200	-0.027	-0.147	-0.119	0.330
TP5-S10	0.625	0.708	0.667	0.750	0.689	0.737	0.749	0.730	0.717	0.896	0.956	0.751	0.095	0.039	0.112	-0.027	0.666
TP4-S11	0.833	0.625	0.708	0.792	0.832	0.720	0.785	0.812	0.376	0.077	0.666	0.396	-0.001	0.134	0.099	0.026	0.743
TP4-S12	0.417	0.458	0.542	0.500	0.558	0.543	0.531	0.529	0.322	0.518	1.000	1.000	0.257	0.160	-0.021	0.056	0.431
TP4-S14	0.750	0.833	0.750	0.833	0.890	0.864	0.903	0.866	0.107	0.523	0.064	0.681	0.160	0.037	0.173	0.039	0.849
TP3-S17	0.792	0.792	0.542	0.625	0.599	0.590	0.590	0.568	0.145	0.072	0.837	0.914	-0.330	-0.351	0.083	-0.102	0.499
TP3-S19	0.708	0.833	0.875	0.708	0.699	0.743	0.754	0.797	0.395	0.500	0.806	0.862	-0.014	-0.125	-0.164	0.113	0.688
TP3-S22	0.792	0.500	0.542	0.708	0.696	0.509	0.462	0.595	0.381	0.680	0.840	0.893	-0.141	0.018	-0.177	-0.196	0.507
TP3-S23	0.875	0.833	0.833	0.792	0.811	0.813	0.794	0.829	0.460	0.736	0.850	0.790	-0.081	-0.026	-0.050	0.046	0.771
TP4-S24	0.708	0.708	0.667	0.667	0.803	0.737	0.711	0.653	0.580	0.870	0.555	0.556	0.120	0.039	0.064	-0.021	0.670
TP4-S25	0.500	0.750	0.667	0.792	0.657	0.732	0.771	0.727	0.283	0.024	0.244	0.991	0.243	-0.025	0.138	-0.091	0.661
TP4-S27	0.696	0.708	0.667	0.667	0.883	0.729	0.674	0.762	0.069	0.592	0.892	0.209	0.216	0.029	0.011	0.127	0.712
TP4-S28	0.708	0.458	0.500	0.500	0.678	0.510	0.480	0.621	0.530	0.717	1.000	0.295	-0.046	0.103	-0.042	0.199	0.504
TP4-S29	0.667	0.583	0.458	0.375	0.684	0.496	0.439	0.370	0.983	0.432	1.000	1.000	0.025	-0.180	-0.046	-0.015	0.407
TP4-S30	0.542	0.583	0.750	0.708	0.738	0.806	0.828	0.866	0.032	0.005	0.726	0.212	0.271	0.280	0.096	0.185	0.768
TP3-S31	0.500	0.500	0.750	0.583	0.505	0.657	0.714	0.555	0.148	0.026	0.987	0.925	0.011	0.243	-0.052	-0.052	0.560
TP4-S32	0.833	0.958	0.708	0.875	0.825	0.805	0.800	0.870	0.905	0.600	0.235	0.636	-0.010	-0.196	0.116	-0.006	0.780
TP4-S33	0.200	0.235	0.333	0.150	0.395	0.811	0.778	0.637	0.032	0.000	0.000	0.000	0.503	0.716	0.579	0.769	0.604
TP3-S36	0.333	0.417	0.292	0.167	0.284	0.337	0.403	0.223	1.000	0.540	0.293	0.299	-0.180	-0.243	0.281	0.258	0.257
TP3-S38	0.000	0.083	0.261	0.083	0.089	0.571	0.546	0.507	0.023	0.000	0.002	0.000	1.000	0.857	0.528	0.839	0.373
TP4-S39	0.000	0.053	0.158	0.045	0.331	0.149	0.245	0.274	0.000	0.083	0.251	0.001	1.000	0.654	0.361	0.837	0.222
TP4-S40	0.625	0.625	0.708	0.958	0.725	0.629	0.698	0.783	0.274	0.167	0.398	0.582	0.141	0.007	-0.016	-0.230	0.660
TP4-S41	0.200	0.143	0.095	0.211	0.553	0.396	0.264	0.437	0.000	0.007	0.006	0.022	0.644	0.645	0.644	0.525	0.363
TP4-S42	0.292	0.583	0.667	0.375	0.311	0.479	0.507	0.439	1.000	0.394	0.216	0.636	0.064	-0.224	-0.324	0.148	0.332
TP3-S49	0.118	0.182	0.333	0.095	0.533	0.818	0.609	0.640	0.000	0.000	0.016	0.000	0.785	0.782	0.462	0.854	0.590
TP3-S51	0.292	0.250	0.208	0.333	0.254	0.263	0.191	0.324	1.000	0.386	1.000	0.333	-0.150	0.052	-0.095	-0.031	0.228
TP3-S56	0.375	0.375	0.500	0.250	0.311	0.370	0.496	0.383	0.548	1.000	1.000	0.115	-0.211	-0.015	-0.007	0.352	0.310
TP4-S57	0.783	0.708	0.625	0.833	0.688	0.782	0.609	0.799	0.979	0.119	0.466	0.867	-0.141	0.096	-0.027	-0.044	0.678
TP4-S58	0.917	0.792	0.750	0.792	0.925	0.837	0.661	0.793	0.797	0.426	0.852	0.565	0.009	0.055	-0.137	0.001	0.765
TP3-S60	0.118	0.381	0.389	0.111	0.611	0.580	0.398	0.475	0.000	0.088	1.000	0.000	0.812	0.348	0.025	0.771	0.450

*FIS—inbreeding coefficient; He—expected heterozygosity; Ho—observed heterozygosity; P-HWE—average P-value of Hardy-Weinberg equilibrium; PIC—polymorphic information content.

The population genetic structure among four geographical populations used in our analysis indicated the power of microsatellite loci developed in our study. Significant population genetic structure indicated low gene flow among different geographical populations. Further study by a dense sampling of different geographical populations may provide new insight into introduction, dispersal and demographical history of *T. palmi*. The markers we have developed here should be useful in a detailed population genetics analysis of *T. palmi*.

Table 3. Null allele frequencies of the 35 microsatellite loci among four populations of *Thrips palmi*.

Locus	Population				Average
	BJDX	HNCS	LNAS	SDSG	
TP3-S01	0.0888	0.0159	0.0123	0.0243	0.0353
TP4-S02	0.0000	0.0000	0.0000	0.0934	0.0233
TP5-S03	0.1602	0.0721	0.0171	0.0253	0.0687
TP4-S04	0.0678	0.0416	0.0729	0.0649	0.0618
TP4-S06	0.0503	0.0490	0.0000	0.0210	0.0301
TP4-S09	0.0000	0.0810	0.0000	0.0000	0.0202
TP5-S10	0.0277	0.0448	0.0000	0.0000	0.0181
TP4-S11	0.0000	0.0000	0.0000	0.0791	0.0198
TP4-S12	0.0000	0.0647	0.0050	0.0368	0.0266
TP4-S14	0.0779	0.0608	0.0248	0.0170	0.0451
TP3-S17	0.0085	0.0000	0.0000	0.0000	0.0021
TP3-S19	0.0000	0.0000	0.0376	0.0000	0.0094
TP3-S22	0.0000	0.0000	0.0000	0.0000	0.0000
TP3-S23	0.0000	0.0000	0.0000	0.0000	0.0000
TP4-S24	0.0000	0.0413	0.0000	0.0000	0.0103
TP4-S25	0.0072	0.0899	0.0000	0.0000	0.0243
TP4-S27	0.0000	0.0953	0.0119	0.0000	0.0268
TP4-S28	0.0000	0.0000	0.0394	0.0137	0.0133
TP4-S29	0.0000	0.0000	0.0000	0.0000	0.0000
TP4-S30	0.0099	0.0866	0.0668	0.1328	0.0740
TP3-S31	0.0000	0.0413	0.0000	0.0860	0.0318
TP4-S32	0.0440	0.0000	0.0000	0.0000	0.0110
TP3-S36	0.0831	0.0000	0.0664	0.0000	0.0374
TP4-S40	0.0000	0.0531	0.0000	0.0000	0.0133
TP4-S42	0.0000	0.0140	0.0417	0.0000	0.0139
TP3-S51	0.0000	0.0000	0.0000	0.0000	0.0000
TP3-S56	0.0000	0.0000	0.1036	0.0000	0.0259
TP4-S57	0.0000	0.0000	0.0000	0.0000	0.0000
TP4-S58	0.0000	0.0000	0.0000	0.0082	0.0020
TP4-S33	0.2432	0.1543	0.2948	0.3144	0.2517
TP3-S38	0.1890	0.1429	0.2903	0.3113	0.2334
TP4-S39	0.1008	0.2762	0.2101	0.1300	0.1793
TP4-S41	0.1743	0.2274	0.1762	0.1910	0.1922
TP3-S49	0.1673	0.2754	0.3306	0.3452	0.2796
TP3-S60	0.0001	0.3032	0.2486	0.1247	0.1691

Table 4. Pairwise F_{ST} values among four populations of *Thrips palmi*.

Population	BJDX	HNCS	LNAS
HNCS	0.0867		
LNAS	0.0298	0.0490	
SDSG	0.0137	0.0527	0.0167

4 Conclusions

In this study, we de novo sequenced a draft genome of *T. palmi* for genome-wide development of microsatellite markers. The is a relatively high abundance of microsatellites in the genome of *T. palmi*. Noel microsatellite loci were developed by initial tests in seven individuals and further evaluation in four natural populations. Population genetics analysis showed structured population in *T. palmi* in China. The stringently selected microsatellite loci in our study provide powerful genetic markers for the genetic study of *T. palmi*.

Funding This study was provided jointly by Promotion and Innovation of Beijing Academy of Agriculture and Forestry Sciences (KJCX20180113), the Natural Science Foundation of Beijing Municipality (6184037), and Beijing Key Laboratory of Environmentally Friendly Pest Management on Northern Fruits (BZ0432).

Acknowledgments We are grateful to Mingliang Li, Qingchao Huang, Zhiyuan Zhao and Xiaoyu Han for their help with the collection of the field populations, Bingyan Li, Mingzhu Chen and Yanjie Lu for their assistance with the molecular works.

References

Antao, T., Lopes, A., Lopes, R.J., Beja-Pereira, A., Luikart, G. 2008. LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, 9: 1–5.

Blacket, M.J., Robin, C., Good, R.T., Lee, S.F., Miller, A.D. 2012. Universal primers for fluorescent labelling of PCR fragments-an efficient and cost-effective approach to genotyping by fluorescence. *Molecular Ecology Resources*, 12: 456–63.

Bolger, A.M., Lohse, M., Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30: 2114–2120.

Bruford, M.W., Wayne, R.K. 1993. Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development*, 3: 939–43.

Cannon, R.J.C., Matthews, L., Collins, D.W. 2007. A review of the pest status and control options for *Thrips palmi*. *Crop Protection*, 26: 1089–1098.

Cao, L.J., Wei, S.J., Hoffmann, A.A., Wen, J.B., Chen, M. 2016a. Rapid genetic structuring of populations of the invasive fall webworm in relation to spatial expansion and control campaigns. *Diversity and Distributions*, 22: 1276–1287.

Cao, L.J., Wen, J.B., Wei, S.J., Liu, J., Yang, F., Chen, M. 2015. Characterization of novel microsatellite markers for *Hyphantria cunea* and implications for other Lepidoptera. *Bulletin of Entomological Research*, 105: 273–284.

Cao, L.J., Wang, Z.H., Gong, Y.J., Zhu, L., Hoffmann, A.A., Wei, S.J. 2017. Low genetic diversity but strong population structure reflects multiple introductions of western flower thrips (Thysanoptera: Thripidae) into China followed by human-mediated spread. *Evolutionary Applications*, 10: 391–401.

Cao, L.J., Li, Z.M., Wang, Z.H., Zhu, L., Gong, Y.J., Chen, M., Wei, S.J. 2016b. Bulk development and stringent selection of microsatellite markers in the western flower thrips *Frankliniella occidentalis*. *Scientific Reports*, 6: 26512.

Chang, N.T. 1991. Important thrips species in Taiwan. *In*: Talekar, N.S. (ed.), *Proceedings of a Regional Consultation Workshop*, Bangkok, Thailand. pp. 40–56.

Du, L.M., Li, Y.Z., Zhang, X.Y., Yue, B.S. 2013. MSDB: a user-friendly program for reporting distribution and building databases of microsatellites from genome sequences. *Journal of Heredity*, 104: 154–157.

Earl, D.A., Vonholdt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359–361.

Evanno, G., Regnaut, S., Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14: 2611–2620.

Jakobsson, M., Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23: 1801–1806.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24: 1403–1405.

Lee, C.E. 2002. Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, 17: 386–391.

Li, H., Lang, K.L., Fu, H.B., Shen, C.P., Wan, F.H., Chu, D. 2015. Analysis of expressed sequence tags (ESTs) from a normalized cDNA library and isolation of EST simple sequence repeats from the invasive cotton mealybug *Phenacoccus solenopsis*. *Insect Science*, 22: 761–767.

Liu, M., Xu, Y., He, J., Zhang, S., Wang, Y., Lu, P. 2016. Genetic diversity and population structure of broomcorn millet (*Panicum miliaceum* L.) cultivars and landraces in China based on microsatellite markers. *International Journal of Molecular Sciences*, 17: 370.

Luo, M., Zhang, H., Bin, S.Y., Lin, J.T. 2014. High-throughput discovery of SSR genetic markers in the mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae), from its transcriptome satabase. *Acta Entomologica Sinica*, 57: 395–400.

Ma, L., Cao, L.J., Gong, Y.J., Hoffmann, A.A., Zeng, A.P., Wei, S.J., Zhou, Z.S. 2019. Development of novel microsatellites for population genetic analysis of *Phenacoccus solenopsis* Tinsley (Hemipeta: Pseudoccoccidae) based on genomic analysis. *International Journal of Biological Macromolecules*, 121: 1135–1144.

Meglecz, E., Costedoat, C., Dubut, V., Gilles, A., Malausa, T., Pech, N., Martin, J.F. 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics*, 26: 403–404.

Peng, Y., Leung, H.C.M., Yiu, S.M., Chin, F.Y.L. 2010. IDBA-a practical iterative de bruijn graph de novo assembler. *Lecture Notes in Computer Science*, 6044: 426–440.

Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945–959.

Rebijith, K.B., Asokan, R., Kumar, N.K.K., Krishna, V., Ramamurthy, V.V. 2007. Development of species-specific markers and molecular differences in mtDNA of *Thrips palmi* Karny and *Scirtothrips dorsalis* Hood (Thripidae: Thysanoptera), vectors of tospoviruses (Bunyaviridae) in India. *Entomological News*, 122: 201–213.

Reitz, S.R., Gao, Y.L., Lei, Z.R. 2011a. Thrips: Pests of concern to China and the United States. *Journal of Integrative Agriculture*, 10: 867–892.

Reitz, S.R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): the making of a pest. *Florida Entomologist*, 92: 7–13.

Reitz, S.R., Gao, Y.L., Lei, Z.R. 2011b. Thrips: pests of concern to China and the United States. *Agricultural Sciences in China*, 10: 867–892.

Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4: 137–138.

Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8: 103–106.

Song, W., Cao, L.J., Wang, Y.Z., Li, B.Y., Wei, S.J. 2017. Novel microsatellite markers for the oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) and effects of null alleles on population genetics analyses. *Bulletin of Entomological Research*, 107: 349–358.

Strassen, R. Zur , 1989. What is *Thrips palmi*? a new quarantine pest in Europe. *Gesunde Pflanzen*, 41(2): 63–67.

van Oosterhout, C., Hutchinson, W.F., WILLS, D.P.M., Shipley, P. 2004. Micro - checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4: 535–538.

Walker, A.K. 1994. A review of the pest status and natural enemies of *Thrips palmi*. *Biocontrol News Inform*, 15: 7N–10N.

Wang, Y.Z., Cao, L.J., Zhu, J.Y., Wei, S.J. 2016. Development and characterization of novel microsatellite markers for the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae) using next-generation sequencing. *International Journal of Molecular Sciences*, 17: 362.

Xiao, S.J., Han, Z.F., Wang, P.P., Han, F., Liu, Y., Li, J.T., Wang, Z.Y. 2015. Functional marker detection and analysis on a comprehensive transcriptome of large yellow croaker by next generation sequencing. *PLoS ONE*, 10: e0124432.

Yang, X.M., Sun, J.T., Xue, X.F., Zhu, W.C., Hong, X.Y. 2012. Development and characterization of 18 novel EST-SSRs from the western flower thrips, *Frankliniella occidentalis* (Pergande). *International Journal of Molecular Sciences*, 13: 2863–2876.

Yi, Q., Liang, S.Y. 2001. *Thrips palmi* were found on protected vegetables in Chaoyang District, Beijing. *China Plant Protection Guide*, 21: 29–29.

Zhou, Y.H., Gu, H.N., Dorn, S. 2010. Isolation of microsatellite loci in the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *Molecular Ecology Notes*, 5: 226–227.