Original article

Genetic differentiation of *Pseudoregma bambucicola* population based on mtDNA COII gene

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ABSTRACT

mtDNA COII gene sequences were identified and analyzed using different types of software, namely, MEGA5.0, DNAMAN, and DnaSP5.0 in four Chinese provinces, namely, Sichuan, Zhejiang, Guizhou and Shanghai. Analysis of molecular genetic variation and its genetic structure and differentiation, combined with NJ tree, MP tree analysis and analysis of molecular variance (AMOVA), at Fst = 0.0582 conclude that the genetic differentiation is low, gene flow is Nm = 8.0911, and gene exchange is sufficient. However, for the geographic populations of *Pseudoregma bambucicola* in the four provinces, their gene exchange is relatively weak at Nm = 0.8284, whereas the genetic differentiation is high at Fst = 0.3764. Based on the data, total nucleotide diversity between the populations is 0.00158 ± 0.00021. The results showed that the total population of Tajima's D and Fu's Fs results are D = −0.885 and Fs = 0.226, respectively. The experimental numerical results showed that this total population is not significant (P > 0.10), indicating that nine different geographic populations are short-term. No expansion occurred in the internal population. This study provided a theoretical and practical basis for the comprehensive prevention and control of *P. bambucicola*.

1. Introduction

Pseudoregma bambucicola, also known as the bamboo stem borer, belongs to the genus *Pseudoregma* of the family *Horimphididae*. *P. bambusicola* is mainly distributed in Thailand, Japan, India and other eastern and southern Asian regions and in Guangdong, Hunan, Zhejiang, Jiangxi and Sichuan in China (Deng et al., 2012). *P. bambusicola* is one of the most serious pests causing damage to bamboo rafts. *P. bambusicola* mainly harms bamboo species, such as *Bambusa multiplex* and *B. multiplexcv* and the *Fernleaf* (Nong et al., 2017). In May and late September, with the increasing and peak number of aphids, bamboo growth greatly affects bamboo surface or causes the appearance of larvae on twigs. The entire bamboo rod was invaded by bamboo pseudoclay, which significantly affected in the survival rate of Hsinchu. *P. bambucicola* secreted honeydew can form stooty mould, which can influence photosynthesis and kill the bamboo. Although *P. bambucicola* poses severe threats to bamboo industry, the genetic analysis of the population structure of *P. bambucicola* is also poorly known. Researching the genetics and likely dynamic evolution can promote our insights about pest survival and pest control.

Recently, most researchers shown that mitochondrial gene sequence analyse genetic evolution, and mtCO I have been used successfully to identify aphid genetic distance inter and intraspecies (Ren et al., 2013). The mitochondrial DNA possess a small molecular weight, high mutation rate, rapid evolution speed and unorganized specificity, and simple structure (Liu et al., 2016). Therefore, mtDNA molecular markers are widely used in insect system evolution and genetic variation. For example, mtDNA molecular marker is used for phylogenetic development, determination of genetic diversity and species identification (Wang et al., 2012; Makoto and Ren, 2014; Yu et al., 2017). The study revealed that based on the CO II gene, the phylogenetic relationships of the genera, species and infraspecific taxa of insects can be well

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resolved (Pruess et al., 2000). At present, the research on the genetic differentiation of mtDNA COII genes of *P. bambucicola* in different geographical populations is still widely unknown. The researcher has study the genetic structure of *P. bambusicola* that sampled throughout southeastern Asia by using mitochondrial DNA sequences, but still lacking of information about the genetic diversity of *P. bambucicola* (Takema et al., 2001).

In the present study, we aimed to used part of mitochondrial COII gene as genetic marker to estimate the population structures of the *P. bambusicola* in nine locations that are all collected from China. Thus our results should provide additional information to genetics of P. bambucicola. In addition, the result should contribute to control of *P. bambucicola* in the bamboo.

2. Materials and methods

2.1. Experimental samples

Based on the area of pest occurrence and its distribution in this study, samples of *P. bambucicola* were collected from nine regions of China in October 2016 (Table 1). A total of 27 aphids were collected from bamboo rods were stored at −20 °C.

2.2. DNA extraction and PCR amplification

All sample from one host bamboo at each site were pooled and used for DNA isolation. The genomic DNA extraction kit from tissues was used to extract DNA from the *P. bambucicola,* which has been ground into powder with liquid nitrogen. The DNA stored at −20 °C. Using the primers (5′-CATTCATATTCAGAATTACC-3′,5′-GAGACCATTACTTGCTTTCAGTCATCT-3′) which were synthesized by Chengdu Engineers Department of Biosynthesis. PCR amplification were performed in a total volume of 50 µL containing, 25 µL 2*Es Taq MasterMix (Taq DNA polymerase, dNTPs, reaction buffer), 1.25 µL of each Primer, 2.5 µL DNA Template, 20 µL DFPC water. PCR amplification reactions is follows: initial denaturation for 2 min at 94 °C, followed by 35 cycles of 30 s each at 94 °C, 30 s at 49 °C, and 40 s at 72 °C, with a final extension for 2 min at 72 °C. The PCR products were identified as 2 µL plus 1 µL 6 × Roading Buffer mix in 120 V at 1.2% agarose gel electrophoresis about 20 min for detection. After the PCR products were electrophoresed in the recovery system, the clear gel was excised and purified by an ordinary DNA gel recovery kit (Beijing Tiangen biotech company), which was then sent to QingKeZiXi Engineers Department of Biosynthesis (Chengdu) for bidirectional sequence (see Tables 2 and 3).

2.3. Data analysis

After calibrating and splicing the determined COII gene sequence, the COII gene was translated into an amino acid sequence to confirm its reliability and accuracy and to prevent the interference of other foreign pseudogenes. The homologous sequence ClustalX software, the download in the GenBank database and the resulting sequences were compared.

Molecular phylogenetic tree of nine different geographic populations of *P. bambucicola* was constructed using neighbour-joining (NJ) method using MEGA5.0 software (Huelsenbeck and Ronquist, 2001). The PCR amplification results of each region were used for 1000 cycles to estimate the phylogenetic tree. The level of self-propagation of the nodes of the evolutionary tree established the phylogenetic tree. PAUP4.0 software was used to build the MP tree, Heuristic search was used to randomly add samples. The experiment was repeated 100 times to build the MP tree (Swofford, 1998; Wang and Zhou, 2016), as shown in Fig. 2. Tajima's D statistical neutrality analysis and calculation of nucleotide polymorphisms were performed using Arlequin 3.0 molecular variation analysis. Pairwise difference model were used for the analysis of molecular variance (AMOVA), was performed based on the population genetic differentiation index (Fst) (Takahata and Palumbi, 1985), gene flow (Nm) (Goldberg and Ruvolo, 1997), specific values and standard values for analysis and comparison. CO II gene sequences were compared using the analysis software of the Vector NTI 10.0 sequence. The base composition (percentage of A, T, C and G) and conserved sites of this sequence were calculated using the software MEGA5.0. Variable sites, number of translations and transversions and calculation of conversion/transversion bias ratios were analysed using MEGA 5.0 software $r = [A * G * k1 + T * C * k2]/[(A + G) * (T + C)]$.

Table 2
Recycling system in the amount of each substance.

Substance	Dosage
Forward Primer	1.25 µL
Reverse Primer	1.25 µL
Template	2.5 µL
DEPCAS	20 µL
Mix enzyme	25 µL

Table 1
Collection data of nine samples of Pseudoregma bambucicola from different geographical populations.

Population code	Sampling sites	Geographical coordinates	Elevation (m)	Collection date
GA	Yuechi County, Guang'an City, Sichuan Province	30.5°N, 106.4°E	387	2016/10/7
NC	Nanchong City, Sichuan Province	30.8°N 106.1°E	306	2016/10/10
DY	Deyang City, Sichuan Province	31.1°N 104.1°E	552	2016/10/6
MY	Mianyang City, Sichuan Province	31.6°N 104.3°E	648	2016/10/4
YB	Yibin City, Sichuan Province	28.8°N 104.6°E	293	2016/10/22
MS	Meishan City, Sichuan Province	29.9°N 104.1°E	419	2016/10/6
HZ	A garden in Huzhou City, Zhejiang Province	30.9°N 120.2°E		2016/10/5
GY	Guizhou University North Campus Library	26.4°N 106.7°E	1090	2016/10/11
SH	Near Shanghai University of Engineering and Technology	31.1°N 121.2°E		2016/10/24

Table 3
PCR amplification conditions.

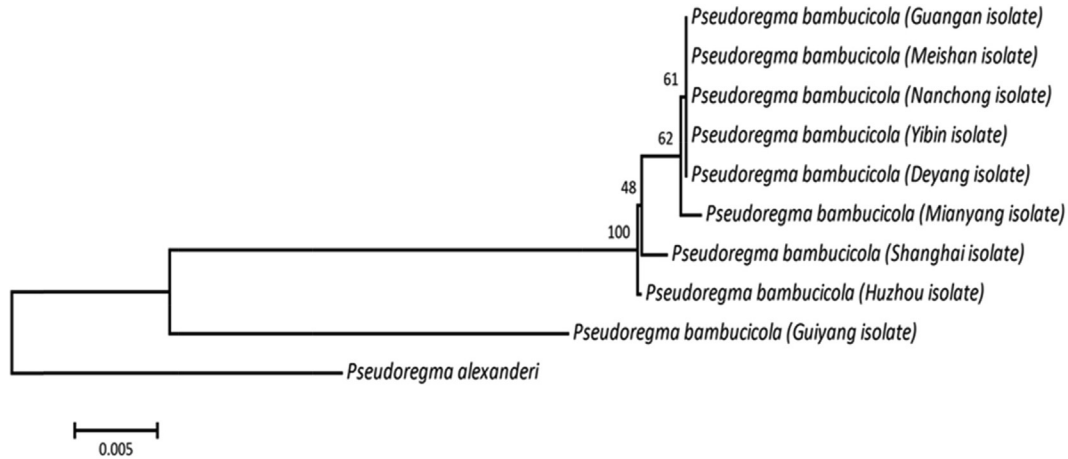| Pre-denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
94 °C 2 min	94 °C 30 s	49 °C 30 s	72 °C 40 s	72 °C 2 min	35

Fig. 1. Neighbour-joining Tree (NJ Tree).
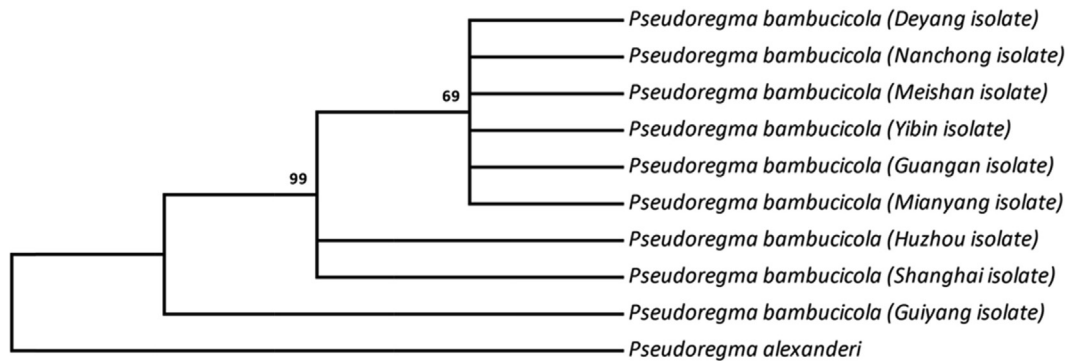

Fig. 2. Maximum parsimony (MP Tree).

3. Results and analysis

3.1. Amplification of the target fragment

After the mtDNA COII gene was amplified by PCR, the sequence fragment obtained after proofreading was approximately 750 bp and was compared with the nucleotide sequence of BLAST in the NCBI website. The homology of the mt DNA COII gene sequences of the bamboo pseudo-anthellae was 99%, which confirmed that these were mt DNA COII gene sequences, and the obtained agarose gel electrophoresis is shown in the Fig. 3 (Li et al., 2015a).

3.2. Phylogenetic analysis of different geographic populations

Using the MEGA5.0 software analysis, the appropriate outgroups were identified first, and the last one was *Pseudoregma alexanderi,* which is the closest species to *P. bambucicola.* For the geographic population, a phylogenetic tree was constructed using NJ. As shown from Fig. 1, the population represented by the nine regions were all independent of the external population and exist independently. The haplotypes represented by the regions were all parallel. The Guangan, Meishan, Nanchong, Yibin, Deyang and
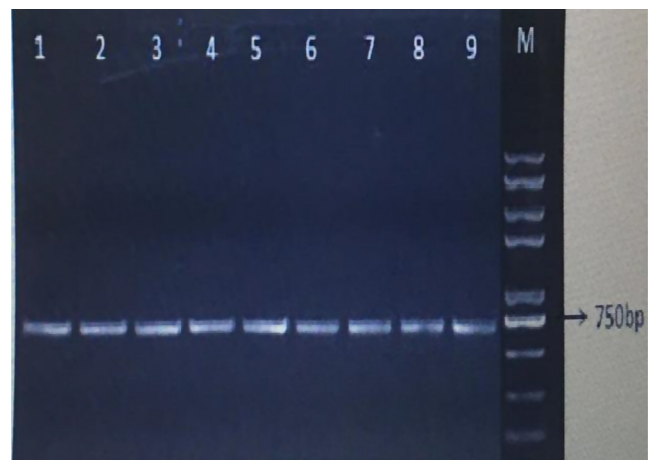

Fig. 3. The above Figure shows the detection map of the gene gel imaging system. The target band is clearly around 750 bp, which is consistent with the gene sequencing result of 738.

Mianyang are clustered together. According to the distribution of population, Mianyang and Shanghai's and Guiyang each are

together as a branch. Guiyang and the other eight regions showed evident branches. Given that the Guangan, Meishan, Nanchong, Yibin, Deyang and Mianyang areas are close in spatial distance, gene exchanges were frequent and the kinship was close, as well as Shanghai, Guiyang and Huzhou were far apart. Geographical factors affected communication between populations and showed slight differences in kinship. Individual of *P. bambucicola* were interspersed among different geographic populations, and therefore did not show obvious geographical lineages.

3.3. COII gene sequence variation analysis

In this experiment, the sequencing results of the gene sequences of several *P. bambusicola* in different regions showed that the COII gene sequence is 738 bp, 723 base sequences and 15 variant sites for the conserved sites were observed. A total of 7 parsimony informative sites were present. Base insertions and deletions were not revealed in the results of the experiment. In these base sequence compositions, the average base compositions A, T, G and C are 41.5%, 38.8%, 11.3%, 8.4%, A + T and G + C contents are 80.3% and 19.7%, respectively. Thus, the content of G + C (19,7%) was lower than that of A + T content (80.3%).

3.4. Genetic variation of COII gene in different geographical populations

The Nm revealed that the genetic communication may occur among various populations. Nm affects the degree of genetic exchange among populations of *P. bambucicola* in different regions by influencing genetic differentiation and weakens the effect of genetic variation within the population. This experiment performed AMOVA on the results of the mtDNA COII gene sequence of the *P. bambusicola* in nine regions. Fst was calculated based on the software DNA sp software, and Nm = (1/F st-1)/2. The Nm value is shown in Table 4.

From the results of molecular variance, the Fst between the six regions of the same province is Fst = 0.0582, and the Fst between the total of nine regions is Fst = 0.3764, and thus the corresponding Nm can be calculated as = 8.0911 and 0.8284, respectively. when 0.05 > Fst > 0, 0.15 > Fst > 0.05 and Fst > 0.15, the degrees of genetic differentiation among populations are relatively weak and very high, respectively (Weight, 1978). The genetic variations among and within the populations of *P. bambusicola* in the same province were 5.8, and 94.2, respectively. The percentages of genetic variation among and within the populations of *P. bambucicola* in nine regions in different provinces were 37.6, and 62.4, respectively. The data that the percentage of variation within the population was evidently greater than the percentage of genetic variation between populations, indicating that the genetic variation of the population of *P. bambucicola* in the nine regions was affected by various factors within the population. When Nm > 4, 4 > Nm > 1 and Nm < 1 indicates that the gene exchanges between the populations are more frequent high and few, respectively (Millar et al., 1991; Li et al., 2015a). This result may be due to genetic drift or other factors. The experiment was similar to the six regions in the province with Nm = 8.0911, indicating that in the same pro-

vince, the genetic exchanges between various populations of *P. bambucicola* were more apparent, and Nm = 0.8284 between the different provinces was relatively small, indicating that geography or the influence of factors, such as weather and climate, blocked the genetic exchange between geographic populations.

3.5. Analysis of genetic diversity and neutrality of COII gene in Pseudoregma bambucicola

This test aims to identify whether the target gene follows a neutral evolutionary model during genetic differentiation. Given that individual suitability was not affected by differences among molecules caused by spontaneous mutation, gene evolution can be caused by the occurrence of genetic drift. Analysis of COII sequences from different geographical populations of *P. bambucicola* by DNA SP 5.0 software is shown in Table 5.

According to the above neutral population test results in the nine regions, most of the geographic populations were not significantly different (P > 0.05), and the evolution showed that the sequences in these populations were consistent with the neutral model (Wang and Xu, 2014). From the above analysis, the table shows that the nucleotide diversity between the populations of *P. bambucicola* in nine individuals was 0.00158 ± 0.00021. According to the results of Tajima's D and Fu's Fs, the total population of Tajima's D and Fu's Fs were −0.885 and 0.226, respectively. P > 0.10 for the two neutral test parameters (Tajima's D and Fu's Fs), indicating that no significant difference among the populations within the population were observed. Therefore, the *P. bambucicola* in China did not exist in the historical period. Population expansion (Harpending et al., 1998) has not been performed in large-scale migration and proliferation phenomenon, at a more stable state at this stage.

4. Discussion

4.1. Analysing population history and genetic structure

The genetic variation of the studied species exhibits a positive effect on revealing the origin and evolution history of the species (Lohman et al., 2008). Non-random distribution is a feature of a species or population under genetic variation. The reason for this non-random distribution may be due to long-term evolution of species, changes in distribution areas, isolation and mutations between groups, destruction of ecological environment, different processes, such as gene Nm, and other similar processes (Anderson et al., 2008; Armstrong and Wratten, 1996). Therefore, an in-depth understanding of the structure of pest populations

Table 4
The result of AMOVA for Pseudoregma bambusicola.

	The same province population	Different provinces
Genetic differentiation index Fst	0.0582	0.3764
Gene Flow N_m	8.0911	0.8284

Note: M is DL8000, and 1–9 are samples of different geographical populations.

Table 5
Genetic Diversity and Neutrality Test of Pseudoregma bambusicola in Different Geographical Populations in Nine Areas of China.

Geographical populations	Nucleotide diversity (π ± SD)	Neutral test and significance test	
		Tajima's D value	Fu's Fs value
Deyang	0.00075 ± 0.00006	1.506	1.582
Nanchong	0.00275 ± 0.00109	0.148	4.724
Meishan	0.00336 ± 0.00069	1.356	4.344
Yibin	0.00048 ± 0.00013	0.594	1.035
Guanghan	0.00201 ± 0.00035	1.072	2.843
Mianyang	0.00077 ± 0.00015	1.214	1.149
Huzhou	0.00330 ± 0.00065	1.336	4.342
Shanghai	0.00372 ± 0.00050	0.552	3.879
Guizhou	0.00055 ± 0.00048	−2.004[*]	1.028
total	0.00158 ± 0.00021	−0.885	0.226

Note: SD stands for standard deviation.
[*] Indicates significant difference (P < 0.05).

helps us to further understand important biological information, patterns of geographic variation and migration patterns (Miller et al., 2003; Timm et al., 2005). In addition, human activities, geographical and reproductive isolations, and the ability of insects to spread affected the population structure of the species (Mendelson and Shaw, 2005). The results of this study indicated that the population history of *P. bambucicola* in the four provinces of China. Their values indicated that significant population expansion in the historical period of the geographical population was not possible.

4.2. Genetic differentiation and distance

In this experiment, in the provinces of MS, DY, YB, GH, MY and NC, the Fst and the Nm were 0.0582 and 8.0911 in each of the six provinces in the same province, respectively.

When Nm > 4, *P. bambucicola* presented frequent genetic exchange among different geographical populations but the genetic differentiation was very low. Although the geographical populations of *P. bambucicola* in different geographic populations in six cities of Sichuan Province in China were constrained by the genetic differentiation among the populations, their gene exchanges were sufficient from the results of nine regions. The Fst and numerical analysis of Nm of the four provinces showed opposite results. This result may be due to geographical and climatic conditions limiting their exchange, especially Guiyang and other geographical populations were relatively distant, genetic exchange was insufficient. In summary, this experimental study provided some direction and molecular evidence for the study of the evolution of *P. bambucicola*.

In conclusions, this study provides first data on the genetic differentiation of *P. bambucicola* in China. The result show that the genetic differentiation of *P. bambucicola* is very low in the same province. This experiment only collected samples from nine areas, most of which were in the Sichuan Basin. Only the genetic differentiation between *P. bambucicola* was shown in some areas, and each sample was collected in only one place of the region, which may cause the samples not to be representative enough. In the future research on P. bambucicola, we will increase the number and extensiveness of samples and conduct, a more in-depth study of the gene sequences of genetic markers at the origin of *P. bambucicola*, so that the results on the differentiation and phylogenetic relationship will provide stronger theoretical basis and better facilitate the understanding of the evolutionary history and population genetic structure of *P. bambucicola* populations.

5. Novelty statement

The research on the genetic differentiation of mtDNA CO II genes of *Pseudoregma bambucicola* in different geographical populations is still widely unknown. These results shown that, this study provided a theoretical and practical basis for the comprehensive prevention and control of *Pseudoregma bambucicola*.

Acknowledgements

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