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# Population genetic structure of *Tyrophagus putrescentiae* in China based on mitochondrial cytochrome oxidase subunit 1 (COI) gene sequences

**Xiao-dong Zhan[1,2], Yi-long Xi[1], En-tao Sun[2], Chao-pin Li[2]**
[1]*College of Life Sciences, Anhui Normal University, Wuhu, China*
[2]*Department of Medical Parasitology, Wannan Medical College, Wuhu, China*

## Abstract

*Tyrophagus putrescentiae* (Schrank) (Acari Acaridae) is an important storage pest mite with a cosmopolitan distribution. Despite of the increasing research on the ecological features and control measure of pest mite, extensive surveys on its population genetics are still relatively fewer. In this study, a total of 127 mitochondrial cytochrome oxidase subunit 1 (COI) gene sequences were used to estimate the population genetic structure of *T. putrescentiae* in China, collected from the stored grain at nine sites. The 88 COI gene haplotypes were defined and grouped into three clades according to the Bayesian phylogenetic trees. No correlation was revealed between their clustering relationships and geographical occurrence. A weak but significant geographic structure was revealed by analysis of molecular variance (AMOVA), and there was no remarkable genetic differentiation among most populations with high level of gene flow, and major genetic differentiation was found within populations. Mantel's test manifested no significant relationship between genetic and geographical distances, which suggested that genetic differentiation was not related to geographic isolation. The pattern of population genetic structure could be attributed to the latest human interference (such as grain trade) and the differentiation of habitat conditions and climate regime.

**Key words:** *Tyrophagus putrescentiae*, genetic differentiation, mite, pest, molecular phylogenetics.

## Introduction

Mites have been attracting much attention because of contaminating grains and acting as carriers of bacteria and toxigenic fungi in the medical and veterinary fields. *Tyrophagus putrescentiae* (Schrank) (Acari Acaridae) is a worldwide distributed storage pest mite, preferring to live in storage grain rich in fat and protein (Kent-Jones *et al*., 1948). A large number of *T. putrescentiae* breeding in stored grain not only lead to the poor grain quality (Malik *et al*., 2018) but also result in disease, such as the dermatitis (Li *et al*., 2003; Hubert *et al*., 2005) and mite allergic diseases (Gamal-Eddin *et al*., 1984; Cui *et al*., 2016). *T. putrescentiae* can use fungi as a food source, and it proved to be an important tool for disseminating both environmental and medically important fungi (Silva *et al*., 2019). Therefore, it is necessary to prevent and control *T. putrescentiae* in storage grain. The use of chemical agents to prevent or control mite infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored product pests. However, pesticides have serious drawbacks, long-term use of pesticides can lead to the human body damage and the pesticide-resistant problem (Bakr and Selim, 2019; Collins, 2012). So, many new methods for mite control have been widely studied.

Genetic structure is one of the most important features of a population, including population genetic diversity, genetic differentiation, population genetic distance, and other parameters that can be used to measure the temporal and spatial distribution of genetic diversity among and within populations. A reliable estimate of the population structure can help people understand the role of different environmental factors and evolutionary forces in determining population dynamics, as well as provide effective information for pest control (David *et al*., 2003; Carbonnelle *et al*., 2007). Understanding gene flow between populations is equally important for elucidating the mechanisms of arthropods resistance (Tsagkarakou *et al*., 1997; Weeks and Hoffmann, 2000). Studies on the population genetic structure of *T. putrescentiae* are rarely reported. The phylogenetic characteristics of *T. putrescentiae* in Korea were reported based on the cytochrome oxidase subunit 1 (COI) in the mitochondrial DNA and the internal transcribed spacer 2 (ITS2) in the robosomal DNA, showing that *T. putrescentiae* was branched into two clusters, the samples in Choi's study clustered together with the known COI sequence of *T. putrescentiae* from China (GenBank accession No. EU078968) although the known COI sequence of *T. putrescentiae* from Korea (GenBank accession No. KJ820777) was separated from the cluster (Choi *et al*., 2017). Although the ITS2 and COI genetic regions are not useful in distinguishing geographically different species, they can help us study low-level phylogenetic relationships between astigmatid mites (Yang *et al*., 2011). The analyses of bacterial communities in *T. putrescentiae* populations by COI and ITS sequences suggested that habitats and food influence the ingested and symbiotic bacteria of *T. putrescentiae* (Erban *et al*., 2016). As reported in the literature (Hutchison and Templeton, 1999), geographic isolation, dispersal (active or passive), pesticide stress, habitat fragmentation, and host factors can influence the evolution and population genetic structure of mites. Evaluating the genetic structure of *T. putrescentiae* populations and its influencing factors is of great significance for studying pesticide resistance.

Because ribosomal DNA internal transcribed spacer (ITS) is cut in DNA transcription, it cannot express proteins or

coding related tissue components, and its nucleotide variation is mostly neutral mutation, which is basically not subject to selection pressure. In this study, we wanted to explore the genetic structure changes of the population of *T. putrescentiae* in the natural selection environment and under the selection pressure of human trade activities. Therefore, mitochondrial COI gene was selected as the molecular marker in this study, and ITS2 was not used. The mitochondrial COI gene is considered to be one of the most powerful tools for evaluating genetic structure and phylogenetic evolution because of its abundant copies, relatively simple structure, rapid evolution rate and maternal inheritance. Use of COI gene can avoid phenomena of genetic promiscuity and cryptogenic hybridization caused by incomplete lineage sorting of nuclear genes. COI genes have been increasingly used to resolve taxonomic status, phylogenetic relationships, and intraspecific differentiation for Acari in recent years (Cruickshank and Thomas, 1999; Navajas and Fenton, 2000; Webster *et al.*, 2004; Erban *et al.*, 2016). Based on the COI sequences, the genetic structure of *Panonychus citri* (McGregor) in China was analysed, and the results showed that there was a weak, but significant, geographic structuring with isolation by distance, which was possibly effected by high level gene flow between some populations (Yuan *et al.*, 2010).

In this study, the population genetic structure, genetic diversity, phylogenetic relationships of haplotypes, and genetic differentiation of *T. putrescentiae* in the stored grain distributed in China were studied using the COI gene as a molecular marker.

## Materials and methods

### Samples

A total of 9 locations were selected from the south to the north of China. The distance of each locations was basically equal. Five sampling sites were selected from each location according to directions of east, west, south, north, and the middle. According to the breeding characteristics of *T. putrescentiae*, the food products (such as rice, millet and wheat flour) were collected from grain warehouses, flour mill, farmer's market and so place in above sites during the period from July to September in the years 2014-2016 (table 1, figure 1). The preliminary expected mites with special morphologic characteristics were observed and separated from these food products under a stereomicroscope. According to the taxonomic descriptions (Hughes, 1976; Li, 2016), the morphological classification (The basal hairs are enlarged and have long teeth; The length of the second dorsal hair is about 2~2.5 times the length of the first dorsal) of mites was conducted under the microscope. Thereafter, the identified mites were stored at −80 °C until processing.

### DNA extraction and amplification

The identified female *T. putrescentiae* individuals after soaking in TE buffer solution (Component: Tris-HCl, EDTA, $H_2O$, pH 8.0) for 2 hours were placed into 200 µL centrifuge tubes containing 20 µL STE buffer solution (Component: NaCl, Tris-HCl, EDTA, $H_2O$, pH 8.0) for completely crushing with a miniature grinding rod which

**Figure 1.** Sampling locations of *T. putrescentiae.* The map shows the central and eastern part of China, and the sampling sites are distributed from north to south. The abbreviations of population name are defined in table 1.

**Table 1.** Collecting locations and dates of *T. putrescentiae* samples.

| Locations | Population | Longitude | Latitude | Date | Monthly mean Temperature °C | Monthly mean Relative Humidity |
|---|---|---|---|---|---|---|
| Wuhu | WH | 118.365109°E | 31.282293°N | Aug 2014 | 28.5 | 82% |
| Fuyang | FY | 115.299189°E | 32.943360°N | Sep 2014 | 22.5 | 70% |
| Suihua | SH | 126.974528°E | 46.660966°N | Jul 2015 | 22.5 | 70% |
| Zhengzhou | ZZ | 113.575440°E | 34.767038°N | Aug 2015 | 26.5 | 71% |
| Beijing | BJ | 116.144111°E | 39.738719°N | Sep 2016 | 20.5 | 68% |
| Shijiazhuang | SJZ | 114.490357°E | 38.017306°N | Sep 2016 | 21.5 | 69% |
| Qianjiang | QJ | 112.906348°E | 30.402899°N | Sep 2016 | 24.0 | 71% |
| Changsha | CS | 113.012023°E | 28.120360°N | Sep 2016 | 25.0 | 73% |
| Chenzhou | CZ | 112.998314°E | 25.803778°N | Sep 2016 | 25.0 | 74% |

was cleaned with 20 μL STE buffer solution after grinding. The grinding fluid was digested at 56 °C for 2 hours with addition of protease K (@ 200 μg/mL) and digested product after digestion was kept for 45 seconds at 95 °C, and then centrifuged for 30 seconds at 2000 (×g) to precipitate residue. The supernatant was used as a template for polymerase chain reaction (PCR) or stored in a −20 °C refrigerator until using. A region of the mitochondrial COI gene of *T. putrescentiae* was amplified with the primers COI-F (5'-TTT GGT GTT TGA TCC GGT A-3') and COI-R (5'-TAA CGA GGC ATA CCA TT-3'), which were designed according to the reported *T. putrescentiae* mitochondrial genome sequence (GenBank accession number: KJ598129). DNA was amplified by polymerase chain reaction in a 25 μL reaction volume containing 2.5 μL 10× buffer, 2.0 μL $MgCl_2$ (2.5 mol/L), 2 μL dNTPs (2.5 mM/L each), 0.5 μL each primer (5 μmol/L), 5 ng template genomic DNA, and 0.4 μL proof reading Taq DNA polymerase (5 U/μL, TaKaRa Ex Taq), with $ddH_2O$ added to 25 μL. The thermal cycling program consisted of denaturation at 94 °C for 5 minutes; followed by 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute; and a final extension at 72 °C for 10 minutes.

### Cloning and sequencing

PCR-amplified products were detected by 1% agarose gel electrophoresis. Target bands were purified with a SanPrep Column DNA Gel Extraction Kit (Sangon Biotech Co. Ltd., Shanghai, China) and then ligated into vector pMD19-T, transformed into *Escherichia coli* (DH5α competent cells) according to the protocol, and identified by blue/white screening. Positive clones were cultured and sent to Sangon Biotech Co. Ltd. (Shanghai, China) for sequencing. In order to ensure the accuracy of the sequencing results, five clones per positive bacterial colony were sequenced in both directions.

### Data analysis

To ensure that the target sequence was the mitochondrial COI gene sequence of *T. putrescentiae*, all sequences were compared using BLAST program and NCBI GenBank database. Sequence alignment was performed using CLUSTAL X1.8 software (Thompson *et al.*, 1997) and supplemented by manual alignment. MEGA 6.0 software (Tamura *et al.*, 2013) was used to examine the presence or absence of a stop codon in the COI sequence and to eliminate mitochondrial COI pseudogenes.

Percentages of sequence differences between pairs of sequences were calculated using DNASTAR1.00 software, and variable sites, polymorphic sites, and parsimony-informative sites were calculated with DnaSP 5.1 software (Librado and Rozas, 2009). The nucleotide diversity (π), haplotype diversity (Hd), and number of haplotypes (N) were also calculated by DnaSP 5.1.

The Bayesian tree was constructed with MrBayes3.1.2 software (Ronquist and Huelsenbeck, 2003). The phylogenetic tree was constructed with the related species *Caloglyphus berlesei* (Michael) (GenBank accession number: KF499016) as an outgroup. The optimal model for the Bayesian tree was tested using the Akaike information criterion (AIC) in Model test 3.7 (Posada and Crandall, 2001). The optimal model was found to be GTR + I. Four Markov chains were run simultaneously, with 3 hot chains and one cold chain, and this was repeated once. A total of $6 \times 10^6$ generations were run and sampled once every 100 generations. The first 25% of the generations were dumped as burn-in, and the residual samples were used to build a consensus tree. A stationary tree was concluded when the average standard deviation of the splitting frequencies was less than 0.01. The confidence factor of respective clade on the Bayesian tree was displayed by the Bayesian posterior probability.

The MEGA 6.0 software (Tamura *et al.*, 2013) was used to compute the mean genetic distances between and within populations, using Kimura 2-parameter model as nucleotide stead model. The genetic distance matrix was calculated using the population comparisons function in the Arlequin 3.1 software (Excoffier *et al.*, 2005) to estimate the genetic differentiation index (F-statistics, $F_{ST}$) between populations. The genetic variation composition and the genetic differentiation index ($F_{ST}$) of the populations were analysed by AMOVA in Arlequin 3.1. The parameters were tested with 1,000 repetitive nonparametric permutations based on pairwise difference. Finally, the correlation between the geographical distance and the $F_{ST}$ matrix was analysed by Mantel's test (Mantel and Greenhouse, 1967) in the Arlequin 3.1 software package with 1,000 replicates to determine significance.

## Results

### Sequence variation and genetic diversity

A total of 127 mitochondrial COI gene sequences were successfully amplified from 127 samples from 9 geographic populations. The length of each sequence was between 1257 bp and 1362 bp. After removing the primers, the final length was 1217 bp. All sequence databases were uploaded to GenBank (Accession No.: MH262418-MH262544) and blasted against the nucleotide database of NCBI to confirm the species of all samples. MEGA analysis showed that there were no base deletions or insertions or stop codons in all sequences, excluding the possibility of a mitochondrial COI pseudogene; therefore, all sequences could be used for subsequent analyses.

By the analyses in molecular software, there were 221 mutation loci, 107 simple informative loci and 217 polymorphic loci, besides 88 haplotypes were defined among the 127 sequences (supplemental material). Finally, 79 haplotypes were observed in only one specimen, while 9 haplotypes were shared by two or more specimens. The number of individuals with shared haplotypes was 37.8% (48/127) of the total number of individuals (table 2). Some haplotypes were shared by multiple geographic populations, while haplotypes in CZ were not shared with other populations. The haplotype ASH5 exhibited the highest frequency, existing 12 individuals distributed in 4 populations: CS, WH, FY, and QJ. The numbers and types of haplotypes found in different populations are shown in tables 2 and 3.

**Table 2.** Haplotypes and numbers of clones among all COI sequences of *T. putrescentiae*.

| Population | n | N | Haplotypes (number of clones) |
|---|---|---|---|
| SH | 7 | 14 | ASH1 (8), H60 (1), H61 (1), H62 (1), H63 (1), H64 (1), H65 (1) |
| BJ | 3 | 3 | ASH2 (1), H2 (1), H3 (1) |
| SJZ | 7 | 14 | ASH2 (8), H66 (1), H67 (1), H68 (1), H69 (1), H70 (1), H71 (1) |
| ZZ | 13 | 17 | ASH6 (1), ASH1 (3), H78 (1), H79 (1), H80 (1), ASH7 (3), H82 (1), H83 (1), H84 (1), H85 (1), H86 (1), H87 (1), H88 (1) |
| FY | 22 | 27 | ASH5 (3), ASH8 (3), H22 (1), H23 (1), H24 (1), H25 (1), ASH4(2), H27 (1), H28 (1), H29 (1), H30 (1), H31 (1), H32 (1), H33 (1), H34 (1), H35 (1), H36 (1), H37 (1), H38 (1), H39 (1), H40 (1), H41 (1) |
| WH | 9 | 9 | ASH5 (1), ASH8 (1), ASH9 (1), H72 (1), H73 (1), H74 (1), H75 (1), H76 (1), H77 (1) |
| QJ | 18 | 24 | ASH5 (7), ASH9 (1), H43 (1), H44 (1), H45 (1), H46 (1), H47 (1), H48 (1), H49 (1), H50 (1), H51 (1), H52 (1), H53 (1), H54 (1), H55 (1), H56 (1), H57 (1), ASH6 (1) |
| CS | 15 | 16 | H4 (1), ASH5(1), H6 (1), H7 (1), ASH3 (2), H9 (1), H10 (1), H11 (1), H12 (1), ASH8 (1), H14 (1), H15 (1), H16 (1), H17 (1), H18 (1) |
| CZ | 3 | 3 | H19 (1), H20 (1), H21 (1) |

n: number of haplotypes; N: number of samples; ASH: a shared haplotype.

**Table 3.** Shared haplotypes among all COI sequences of *T. putrescentiae*.

| Shared haplotype | Clone |
|---|---|
| ASH1 | SH103, SH106, SH115, SH117, SH4, SH5, SH67, SH7, ZZ146, ZZ55, ZZ65 |
| ASH2 | BJ3, SJZ12, SJZ13, SJZ27, SJZ53, SJZ57, SJZ58, SJZ69, SJZ70 |
| ASH3 | CS35, CS4 |
| ASH4 | FY15, FY9 |
| ASH5 | WH21, CS13, FY13, FY24, FY27, QJ5, QJ59, QJ57, QJ23, QJ25, QJ46, QJ56 |
| ASH6 | QJ60, ZZ33 |
| ASH7 | ZZ143, ZZ151, ZZ54 |
| ASH8 | CS42, FY11, FY20, FY28, WH6 |
| ASH9 | QJ12, WH16 |

### Population genetic structure

Based on the COI gene sequences, phylogenetic trees were constructed using the Bayesian method (figure 2). Clustering analysis showed that 88 haplotypes were divided into 3 groups (clade I, clade II, and clade III). Clade I included 5 geographical populations (eight FY haplotypes, three CS haplotypes, one CZ haplotype, one WH haplotype, and two QJ haplotypes). Clade II included 8 geographical populations (nine CS haplotypes, seven FY haplotypes, two CZ haplotypes, twelve QJ haplotypes, two WH haplotypes, one BJ haplotype, six SJZ haplotypes, and six ZZ haplotypes). Clade III consisted of 3 geographic populations (one BJ haplotype, six SH haplotypes, and four ZZ haplotypes). Percent of sequence differences (By Jotun Hein Method) among all 88 haplotypes ranged from 0 to 7.1%, with a mean of 2.9%. Ranges for clades I, II, and III were 0-7.1%, 0.1-5.8%, and 0-6.9%, respectively.

In this study, the Hd and $\pi$ of the nine geographic populations were 0.978 ± 0.006 and 0.02859% ± 0.00195%, respectively. Among all populations, the Hd values of SJZ and SH were lowest (0.692 ± 0.137). The $\pi$ ranged from 0.00117% to 0.03944%, with the lowest in the SH population and the highest in the CZ population. The $\pi$ of each population is given in table 4.

The genetic distance within populations based on COI sequences ranged from 0.0012 to 0.0416, which was the lowest (0.0012) in the SH population and the highest (0.0416) in the CZ population. The genetic distances between populations were between 0.011 and 0.049 with lowest between the QJ-SJZ populations and highest between the WH-ZZ and SH-FY populations (table 5).

The AMOVA showed that the genetic differentiation between the SH population and the other populations was extremely significant ($P < 0.01$), which also can be find in the SJZ population. For the WH and ZZ populations, there were significant differentiation between them and most of the other populations. The $F_{ST}$ values for 3 pairs of populations, BJ-CZ, CS-CZ, and FY-CZ, were the lowest and were negative ($F_{ST} < 0$), indicating a high amount of gene flow between these pairs of populations (table 6). AMOVA showed that most of the variations observed within the populations, accounting for 63.9% of the total variations. The remaining variations came from the inter-populations (36.1%). Average $F_{ST}$ between populations was 0.36153, showing that there was significant genetic differentiation among different geographic populations ($P < 0.01$).

The geographical distances and $F_{ST}$ values among nine populations are shown in table 6. Results of Mantel's test showed that the geographical distances between populations were not related to the genetic differentiation index (correlation coefficient $r = 0.5553$, $P = 0.067$), which indicates that geographic isolation is not a main contributor to the genetic differentiation of *T. putrescentiae*.

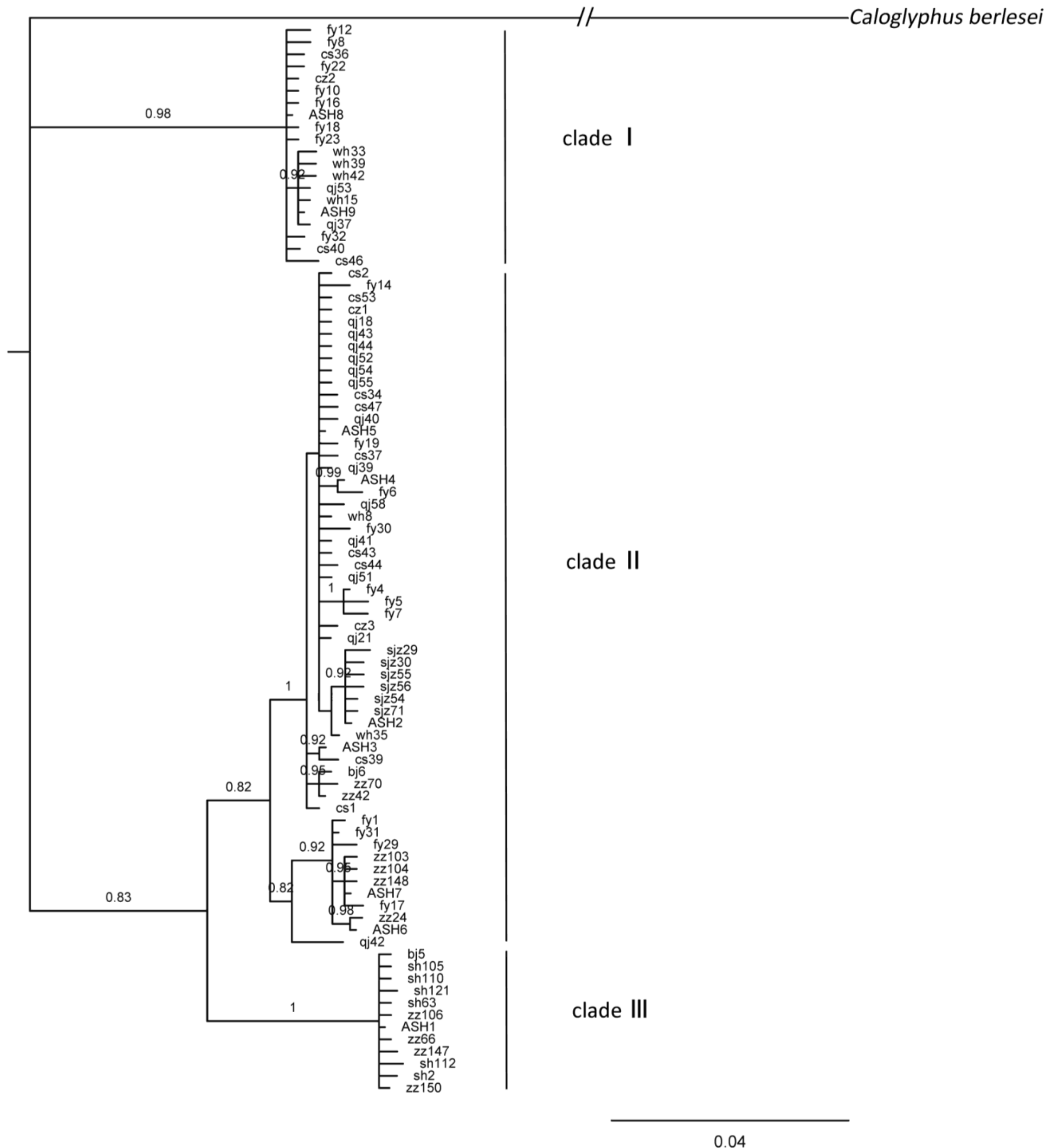


**Figure 2.** Bayesian phylogenetic tree of *T. putrescentiae* based on COI gene sequences. The abbreviations of population name are lowercase. Clustering analysis showed that 88 haplotypes were divided into 3 groups (clade I, clade II, and clade III).

**Table 4.** Haplotype diversity (Hd) and nucleotide diversity (π) for each population of *T. putrescentiae* based on COI sequences.

| Population | N(number of haplotypes)/N(number of samples) | Hd (h ± SD) | π ± SD |
|---|---|---|---|
| SH | 7/14 | 0.692 ± 0.137 | 0.00117 ± 0.00036 |
| BJ | 3/3 | 1.000 ± 0.272 | 0.02356 ± 0.02356 |
| SJZ | 7/14 | 0.692 ± 0.137 | 0.00129 ± 0.00039 |
| ZZ | 13/17 | 0.956 ± 0.037 | 0.01930 ± 0.00161 |
| FY | 22/27 | 0.980 ± 0.017 | 0.03199 ± 0.00197 |
| WH | 9/9 | 1.000 ± 0.052 | 0.02976 ± 0.00709 |
| QJ | 18/24 | 0.924 ± 0.049 | 0.01572 ± 0.00559 |
| CS | 15/16 | 0.992 ± 0.025 | 0.02472 ± 0.00629 |
| CZ | 3/3 | 1.000 ± 0.272 | 0.03944 ± 0.01783 |

**Table 5.** Average genetic distances within and between *T. putrescentiae* populations based on COI sequences.

| Population | BJ | CS | CZ | FY | QJ | SH | SJZ | WH | ZZ |
|---|---|---|---|---|---|---|---|---|---|
| BJ | **0.0242** | | | | | | | | |
| CS | 0.026 | **0.026** | | | | | | | |
| CZ | 0.030 | 0.027 | **0.0416** | | | | | | |
| FY | 0.036 | 0.031 | 0.031 | **0.0337** | | | | | |
| QJ | 0.020 | 0.021 | 0.025 | 0.029 | **0.0165** | | | | |
| SH | 0.023 | 0.042 | 0.045 | 0.049 | 0.038 | **0.0012** | | | |
| SJZ | 0.014 | 0.018 | 0.023 | 0.030 | 0.011 | 0.035 | **0.0013** | | |
| WH | 0.047 | 0.037 | 0.035 | 0.034 | 0.039 | 0.057 | 0.043 | **0.0314** | |
| ZZ | 0.021 | 0.031 | 0.034 | 0.038 | 0.025 | 0.021 | 0.021 | 0.049 | **0.0199** |

The numbers on the diagonal represent the average genetic distance within each population, and the numbers below the diagonal represent the average genetic distances between populations.

**Table 6.** Geographic distance (below diagonal; Unit: kilometre) and $F_{ST}$ values (above diagonal) among *T. putrescentiae* populations. (* $P < 0.05$, ** $P < 0.01$)

| Populations | BJ | CS | CZ | FY | QJ | SH | SJZ | WH | ZZ |
|---|---|---|---|---|---|---|---|---|---|
| BJ | 0 | 0.01929 | −0.09873 | 0.13431 | 0.05408 | 0.76129* | 0.47997* | 0.37697 | −0.03073 |
| CS | 1325.02 | 0 | −0.19643 | 0.02326 | 0.00107 | 0.66589** | 0.23157** | 0.22854 | 0.25625** |
| CZ | 1578.59 | 257.879 | 0 | −0.17643 | −0.03625 | 0.81585** | 0.52226** | −0.00205 | 0.22102 |
| FY | 760.207 | 579.887 | 825.427 | 0 | 0.14179* | 0.57406** | 0.33538** | 0.04754 | 0.26936** |
| QJ | 1080.06 | 254.288 | 512.037 | 362.412 | 0 | 0.72960** | 0.17608** | 0.43336** | 0.28276** |
| SH | 1166.90 | 2396.87 | 2630.02 | 1820.05 | 2178.03 | 0 | 0.96386** | 0.76387** | 0.46748** |
| SJZ | 239.268 | 1110.24 | 1366.81 | 569.541 | 860.027 | 1404.32 | 0 | 0.67921** | 0.48296** |
| WH | 962.52 | 625.792 | 804.318 | 343.142 | 530.794 | 1864.28 | 829.227 | 0 | 0.48902** |
| ZZ | 598.323 | 741.812 | 999.298 | 258.052 | 489.84 | 1736.06 | 370.968 | 591.724 | 0 |

## Discussion

Although distributed widespread in the world, the mite populations usually diverge from each other because of the nature of island-like and fragment habitats, so resulting in the high genetic diversity. In this study, although most mitochondrial COI haplotypes represented population-specific origin, some haplotypes were shared by multiple populations. For example, shared haplotype ASH8 was present in the WH, FY, and CS populations. This phenomenon that populations with shared haplotypes in large geographic distance may be due to gene flow between populations caused by human trade activity or retained from common ancestry (Posada and Crandall, 2001). The above populations of WH, FY and CS all came from important food distribution centres in China, and the amount of food trade was very large. *T. putrescentiae* can flow in these three regions along with the transportation of grains, which may lead to the high level of gene flow among *T. putrescentiae* populations in these sampling sites. In addition, sequence analysis showed that there were much differences in nucleotide diversity ($\pi$) within each of nine geographic populations, with the values of $\pi$ being highest in CZ population and lowest in SH population. The occurrence of monomorphic populations might be due to the small sample sizes (there were only 3 samples from the Chenzhou geographic population), but could also be caused by high rates of inbreeding. High level of population genetic diversity based on COI gene sequences has also been recorded in other mite species, suggesting that high level genetic diversity at this locus may be common in the fauna of mites (Osakabe *et al*., 2005; Skoracka and Dabert, 2010; Yang *et al*., 2011; Sakamoto *et al*., 2014). The genetic diversity of *Euseius nicholsi* (Ehara et Lee) (Phytoseiidae) based on the ITS and COI sequences among 10 populations showed the high levels of haplotypes and nucleotide diversity in most of the populations, especially in Chengdu and Chongqing populations collected from southwestern China (Yang *et al*., 2012). In our study, the nucleotide and haplotype diversities of the southern population were higher than those of the northern population, which was consistent with the above findings. The pattern of population genetic diversity could be attributed to the differentiation of habitat conditions and climate regime.

The Bayesian trees based on the mitochondrial COI gene sequences showed that haplotypes of the same geographic population were not clustered together but scattered throughout the tree, with shared haplotypes among different geographic populations (figure 2). Similarly, Mantel's test based on COI gene sequences showed that among the nine *T. putrescentiae* populations, the geographic distance indicated no significant correlation with the genetic differentiation index, and the results were consistent with the findings reported by Murillo *et al*. (2018), which suggested that geographical isolation might not be the main cause of genetic differentiation among *T. putrescentiae* populations. Although *T. putrescentiae* is small and wingless, with limited migration /dispersal abilities, it didn't make significant contribute to genetic differentiation. Alternatively, grain trade in human activities could responsible for the wide spread

of this mite species which dwells in the stored agriculture products. In comparison, *E. nicholsi* showed high levels of genetic diversity and significant differentiation among geographical populations (Yang *et al*., 2012), as *E. nicholsi* is an field agricultural mite and its population is relatively fixed. So the difference of habitat mode might result in divergent pattern of genetic differentiation.

Analysis of molecular variance based on COI gene sequences showed that the genetic differentiation among the populations was significant and that genetic variation mainly came from within rather than between populations. Genetic differentiation analysis showed that differentiation between the SH population and the other populations was extremely significant, which also can be find in the SJZ population. For the WH and ZZ populations, there were significant differentiation between them and most of the other populations. *T. putrescentiae* often breed in stored grain (Hughes and Maunsell, 1973), and can easily spread passively with human food trade (Palyvos *et al*., 2008). Theoretically, considering the geographic segregation and environmental heterogeneity, populations of *T. putrescentiae* should differentiate among geographical population. However, gene flow was elevated between geographic locations with frequent food trade. This could explain why significant genetic differentiation were observed between each of SH, SJZ, WH, and ZZ populations and the other populations, and high levels of gene flow occurred between BJ, CS, CZ, FY, and QJ populations at the same time. These results suggest that geographic isolation and food trade have significant impacts on the population genetic structure of *T. putrescentiae*, as suggested in previous studies (Yuan *et al*., 2010; Yang *et al*., 2012). In summary, we assume that limited gene flow lead to genetic differentiation in *T. putrescentiae* populations at the stage of its early evolutionary history, and the spread of *T. putrescentiae* among differentiated populations might due to the recent grain trade by humans, thus leading to high levels of gene flow among them.

In conclusion, the high levels of genetic diversity and gene flow among *T. putrescentiae* populations were detected, which could be attributed to the grain trade in human activities, resulting in the wide spread of this mite species. Considering the interference of human activities to the distribution of *T. putrescentiae* populations, people should pay attention to the species identification and the grain trade in order to develop effective management in pest mites.

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**Authors' addresses:** Yi-long XI (corresponding author: ylxi1965@126.com), Xiao-dong ZHAN, College of Life Sciences, Anhui Normal University, Wuhu, 241000, China; En-tao SUN, Chao-pin LI, Department of Medical Parasitology, Wannan Medical College, Wuhu, 240002, China.

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