Polymorphisms of Mitochondrial *COII* and16S rRNA Gene in *Meloidogyne enterolobii* on Mulberry

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Abstract

This study explores the genetic diversity and polymorphisms of *Meloidogdyne enterolobii* (*M enterolobii*) on mulberry in China. The sequence of cytochrome oxidase subunit II (COII) and 16S rRNA gene in *M enterolobii* populations in Guangdong, Guangxi, and Hunan Provinces were PCR-amplified, sequenced, and analyzed for genetic diversity. The haplotypes (Hap) numbers, the total number of mutations, the average number of nucleotide differences (K), haplotype diversity (Hd), and nucleotide diversity (π) of *mtCOII* gene were 14, 25, 3.563, 0.942 and 0.00429, respectively. The significant differences in Fst value (0.125) and a high level of gene flow (2.83) were detected among the 19 *M enterolobii* populations. High genetic variation within each population and a small genetic distance among populations was observed. Both phylogenetic analyses and network mapping of the 14 haplotypes revealed a dispersed distribution pattern of the 19 *M enterolobii* populations. There was an absence of branches strictly corresponding to the 19 range sampling sites. The analysis of molecular variance (AMOVA) revealed that the genetic differentiation of *M enterolobii* populations was mainly contributed by the variation within each of the defined geographical groups. No significant correlation was found between the genetic distance and geographical distance of 19 *M enterolobii* populations. This study provides theoretical basis for the future control of *M enterolobii* and also provides a guarantee for the production of other hosts of *M enterolobii*.

Introduction

The root-knot nematode (RKN) *Meloidogyne enterolobii* was first discovered on *Euterolobium contortisiliquum* in Zhangzhou, Hainan Province, China (Yang & Eisenback, 1983). It is a highly virulent and pathogenic pest with a wide host range (Wang et al., 2015), causing severe damage to agricultural crops (Chen et al., 2016).

Its most economically important hosts include *Glycine max* Merr., *Zea mays* L., *Gossypium* spp., *Nicotiana alata* Link et Otto, *Psidium guajava* Linn., *Litchi chinensis* Sonn., *Citrullus lanatus*, *Lycopersicon esculentum* Mill., *Ipomoea batatas* Lam., *Capsicum annuum* L., *Vigna sinensis* Sav., *Cajanus cajan* Millsp., *Cucurbita moschata*, *Aquilaria sinensis* Spreng., *Syzygium aromaticum*, *Ziziphus jujuba* Mill., *Piper nigrum* L., *Cucumis melo* Var. *saccharinus*, *Ipomoea aquatica* Forssk, and *Morinda citrifolia* (Liao, 2001; Brito, 2007; Rammah, 1988). Paestakahashi et al. (2015) were the first to report parasitic damage caused by *M enterolobii* on mulberry in Brazil. Subsequent morphological and molecular analyses conducted by Zhang et al. (2020) identified *M enterolobii* as the pathogen responsible for mulberry infections in the Guangdong Province.

In recent years, *M enterolobii* has gradually spread from the south to the north of China (Wu et al., 2019). It has also been found in Africa, America, and Europe (Onkendi et al., 2013). Currently, *M enterolobii* is considered one of the most threatening pathogenic nematodes in tropical and subtropical regions throughout the world, with an estimated potential yield loss of 20% (Zhuo et al., 2008). Chemical treatments remain the most commonly used measured to control *M enterolobii* infections. However, the overuse of such chemicals has led to the development of pesticide resistance. Additionally, pesticides residues pose potential health risks to humans (Kaplan, 2004). Therefore, the better understanding of the genetic characteristics of *M enterolobii* may identify potential resistance genes and novel approaches to control *M enterolobii* infections.

Ribosomal DNA (rDNA) is widely used as a molecular marker to identify and phylogenetically characterise different nematodes. Notably, rDNA PCR-based approaches have been employed to identify *Bursaphelenchus* spp (Jiang et al., 2005). and *Pratylenchus* spp. (Mizukubo et al., 2007). Mitochondrial DNA (mtDNA) is another emerging tool for the genetic and taxonomic characterisation of plant parasitic nematodes due to its small molecular weight, high stability, and relatively conserved gene composition (Duan, 2013). Sun et al. (2005) used the mitochondrial cytochrome oxidase subunit II (*COII*)-*LrRNA* gene fragment to distinguish between different *Meloidogyne* spp. Deng et al. (2016) used the *COII* gene to analyse the genetic diversity of the *Rotylenchulus reniformis* population. Janssen et al (2016) performed a haplotype-based mtDNA analysis in *Meloidogyne* and found that certain mitochondrial haplotypes were associated with specific esterase isozyme patterns, suggesting that different parthenogenetic lineages can be identified using mitochondrial haplotypes. Rashidifard (2019) analysed the molecular characteristics of 37 *Meloidogyne* populations from four provinces in South Africa and found that *COII-16S* could accurately identify different *M enterolobii* populations. Additionally, *COII* and *16S* rRNA characterisation has been proven useful for the identification of different *Meloidogyne* species from different geographic regions (Onkendi & Moleleki, 2013). Ye et al. (2007) used the sequence of cytochrome oxidase subunit I (*COI*) and other genes to construct 19 phylogenetic trees of *Bursaphelenchus* spp., and analysed the phylogenetic relationships among the species of the genus. Therefore, mitochondrial genes can be used to analyse the evolution and genetic diversity of plant nematodes.

In this study, we analysed the genetic diversity of *M enterolobii* collected from mulberry trees in southern China, Guangdong, Hunan, and Guangxi. Phylogenetic analysis was performed based on the cytochrome oxidase subunit II (*COII*) gene, partial cds; tRNA-His gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial.

Materials And Methods

Nematode collection: The nematode samples in this experiment were collected from the main sericulture areas in Guangdong, Guangxi, and Hunan Provinces, China (Table 1). The 19 populations were classified into three groups: YB (Northern Region of Guangdong Province, China), YN (Southern Region of Guangdong Province, China) and YZ (Central Region of Guangdong Province, China). This distribution rule is based on the geographical distribution and climatic zone characteristics of the *M enterolobii* populations in Guangdong Province.

Nematode extraction

Female nematode was extracted from root-knot samples using the method described in Liu et al. (2020).

PCR and sequencing

A total of 19 populations, previously identify as *M enterolobii* based on rDNA-ITS sequencing, and maintained in the lab were used in this study. Ten females of each population were placed into 5 µL of worm lysis buffer (WLB) containing proteinase K for DNA extraction (Williams et al., 1992). The DNA samples were stored at -20°C.

The primers C2F3 (5'- GTCAATGTTCAGAAATTTGTGG – 3') and MRH106 (5'- TAATTTCTAAAGACTTTTCTTAGT – 3') were used for the cytochrome oxidase subunit II (COII) gene, partial cds; tRNA-His gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial (Stanton et al., 1997). The 25 µL PCR mixture contained 12.5 µL 2× PCR buffer for KOD FX (TOYOBO), 5 µL 2 mM dNTPs, 1 µL of each primer (10pmol/µL), 20 ng of the isolated DNA, and distilled water. The PCR was carried out in a lab cycler (Applied Biosystems) under the following conditions: initial denaturation at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 2 min; and a final extension at 72°C for 10 min.

All PCR products were separated by electrophoresis on a 1% TBE agarose gel. The amplified products were sequenced (BGI Genomics, BGI-Shenzhen) and the haplotypes were calculated using DNASP 5.0. The sequences obtained were submitted to GenBank and get accession number.

Genetic diversity analysis: The sequencing chromatography was analyzed by Chromas 2.3 (https://chromas.updatestar.com/), and the DNA sequences were analyzed using the Seq Man program in DNAstar 6.0 (DNASTAR, Madison USA)

(1) Sequence analysis

The sequence within the *mtCOII* gene were viewed using Clustal X 1.83. According to Librado & Rozas (2009), we calculated the percentage of sequence variation, nucleotide diversity (π), haplotype diversity (Hd), mitochondrial DNA (Dxy), Fst values, Gst values, and the average number of nucleotide changes (K) of the 19 *M enterolobii* populations was calculated. Gene flow (Nm) between populations was geted using the mitochondrial-specific gene formula Fst = 1/(1 + 2Nm) (Takahata & Palumbi, 1985).

(2) Neutrality test

According to Tajima (1989) and Kimura (1983), the DNA fragments were subjected to Tajima's D and Fu's Fs neutrality test in the population and group levels using Dna SP 6.0 (Kimura, 1983; Tajima, 1989).

(3) Haplotype analysis

Base content and polymorphic loci were analyzed by MEGA 7.0 according to a previously reported method (Librado & Rozas, 2009). The variability between sequences was calculated based on the Kimura-2-Parameter (K2P) model, and a Neighbor-Joining (NJ) phylogenetic tree was constructed using MEGA 7.0. NETWORK 5.0 based on the Median-joining method drew the haplotype network diagram.

(4) Molecular variation analysis

The genetic distance between populations was figured up using the MEGA 7.0, and the AMOVA molecular variance components and haplotype frequencies were analyzed using the Arlequin 3.5. The correlation between genetic distance and geographic distance was calculated using SPSS (22.0).

Results

Sequence and variation analysis of the *mtCOII* gene fragment in *M. enterolobi populations*

A total of 19 homologous sequences of the *mtCOII* gene were amplified by PCR. Each sequences of 19 *M enterolobii* populations all were 831bp which used for genetic analysis. The accession numbers of 19 *M enterolobii* populations is shown in the Table2. There are 15 polymorphic loci (1.8% of the total number of bases analyzed), 6 S-singleton sites and 9 parsimony-informative sites, which accounted for 40% and 60% of the total polymorphisms identified, respectively. The S-singleton sites were located at positions 566, 647, 669, 675, 763, and 798 of the *mtCOII* gene fragments, and the parsimony-informative sites were located at positions 64, 65, 103, 465, 661, 678, 720, 727, 731, respectively. The contents of a, t, c and g were 48.99%, 36.37%, 10.68%, and 3.96%, respectively, and the content of a + t was 85.36%, showing a significant a/t bias. The total number of mutations detected in the *M enterolobii* populations was 25, and the conversion/transversion rate R was 0.9.

Nucleotide and haplotype diversity analysis of M enterolobii populations based on mtCOII gene

The number of variable sites, average number of nucleotide differences (K), haplotype diversity (Hd), and nucleotide diversity (π) of *mtCOII* gene in *M enterolobii* populations were 25, 3.563, 0.942 (> 0.5) and 0.00429. Tajima's D and the Fu's Fs values were-1.26569 and − 9.072, respectively, indicating that all populations underwent neutral selection, and the changes in population were not significant.

Among the 14 haplotypes identified (Table 3), Hap9 was found in 5 populations with a frequency of 26.36% (MN907173、MN9071715、MN907179、MN9071710、MN9071716); Hap14 appeared in 2 populations (MN9071712、MN9071717) with a frequency of 10.52%. Other haplotypes were identified only once in the tested populations.

Phylogenetic analysis of M enterolobii populations based on mtCOII gene

We selected sequences of *M. enterolobii* from different hosts in NCBI and 19 *M. enterolobii* sequences in this study for phylogenetic analysis. The phylogenetic tree constructed by the neighborhood-joining method showed that the 19 *M enterolobii* populations are on the same big branch with 96% bootstrap support and the other sequences selected on NCBI gather on another big branch with 98% bootstrap support. The out-group (*C. elegans*) is far away other branches (Fig. 1). The 19 *M enterolobii* populations were distributed in two evolutionary clades, among which Clade A with 12 populations took the main part while only 7 populations appeared in Clade B (Fig. 1). MN9071716, MN907179, MN9071710, MN9071715 and MN907173 lie in the same clade, in which there was no obvious grouping among populations in this clade, and the similarity among all the populations reached 100%. Additionally, MN9071712 and MN9071717 also lie in the same clade with the similarity of 100% among the populations. Other populations had their own independent haplotypes (Fig. 1). Based on the phylogenetic tree, it was found that the haplotypes of 19 populations were scattered free without any obvious geographical group distribution pattern. Therefore, it is demonstrated that *M enterolobii* populations in China have abundant mitochondria Hd and π, suggesting that the nematode populations have stronger adaptability to the changes in external environment.

Haplotype mediation network map of M enterolobii populations based on mtCOII gene

An intermediary network of *M enterolobii* populations was constructed by NETWORK 5.0 based on the Median-joining method. As shown in Fig. 2, Hap9 was shared by seven geographic populations (MN907173、MN9071715、MN907179、MN9071710、MN9071716). Hap14 occurred in two populations (MN9071712、MN9071717). The other haplotypes occur only once, corresponding to a single geographic population. This network could clearly explain the evolutionary relationships between each haplotype and the distribution of each geographical group, further supporting the phylogenetic tree (Fig. 2).

Genetic distance analysis of M.enterolobii populations based on mtCOII gene

The genetic distances among different *M enterolobii* populations were calculated based on *mtCOII* sequences using MEGA 7.0 (Appendix 1). The results showed that the genetic distances between various groups ranged from 0.000 to 0.011. YL and MM, QQ, SS, KB; MM and QQ, SS, KB; QQ and SS, KB; ZS and ZS1; and the genetic distance between the SS and KB populations had the smallest genetic distance (0.000), whereas the genetic distance between the YD and GB population was the greatest (0.011). However, the genetic distances between different populations varied little.

Correlation between geographic distance and genetic distance in *M. enterolobi ipopulations based on mtCOII* gene

The correlation between genetic distance (Appendix 1) and geographic distance were investigated (Appendix 2) based on *mtCOII* gene (Fig. 3). The results showed that there was no significant correlation between the genetic distance and the natural logarithm (LN km) matrix ($r = 0.093$, $p=|-0.204|>0.05$) of the geographic distance among samples collected, indicating that geographical distance is not the main factor leading to root-knot nematode populations differentiation.

Genetic variance of the M enterolobii groups based on mtCOII gene

Based on the AMOVA method, Arlequin software was used to analyze the genetic variation between *M enterolobii* groups *in* Guangzhou, Hunan and Guangxi Province in China. The intra-population differentiation parameter FST was 0.02004 (P < 0.0001) (Table 4). The variations within groups accounted for 98% of total variation, and the variations among groups accounted for 2% of total variation. These results indicated that the genetic differentiation of the root-knot nematode populations was mainly due to the variations within each group rather than those among different groups.

Nucleotide and haplotype diversity analysis of M enterolobii groups based on mtCOII gene

The number of haplotypes detected in the YB, YZ and YN groups was 4, 7, and 4 (Table 5), and the haplotype diversity Hd values among the YB, YZ, and YN groups were all close, 0.900, 0.867 and 1.000, respectively, indicating that the three groups are rich in haplotype diversity. The highest nucleotide diversity of the YN group is 0.0470, and the lowest nucleotide diversity of the YZ group is 0.00209. The sequence is YN > YZ > YB, indicating that there are differences in nucleotide diversity among the three groups. The values of the Tajima's D and Fu's Fs of the three groups conform to the law of neutrality, and the group changes are not significant.

Genetic differentiation and gene flow analysis of the M enterolobii groups based on mtCOII

The total Fst value and gene flow (Nm) value of 19 *M enterolobii* populations was 0.125 (P < 0.15) and 2.83 (P > 1). We observed the highest gene exchange rate between YB and YZ (Nm = 4.5) (Table 6), whereas the gene exchange rate between YZ and YN was the lowest (Nm = 1.94). The Kxy values (sum/individual number) between YZ and YB, YN groups are similar to 4.14743 and 4.04762, respectively; the minimum Kxy value between YB and YN groups is 2.93333.

Discussion

In this study, we assessed the genetic diversity of *M enterolobii* from different geographic regions in China by analysing the sequence of the mtDNA gene *COII*. We observed a moderate genetic diversity among the populations from different collection sites (Fst = 0.125, Nm = 2.83), suggesting gene exchange between these populations. The overall genetic variation in *M enterolobii* was primarily caused by variation within rather than between different geographic groups. We found no significant correlation between genetic distance and geographic distribution. This study enriches the phylogenetic information of *M enterolobii*, and provides the basic evidence for the inherent genetic factors of damage from *M enterolobii*.

Genetic diversity is not only the basis of biodiversity but also a driving force for the evolution of species. The reduction or loss of genetic diversity is a great threat to a population or species living in a changing environment. Haplotype polymorphisms (Hd) and nucleotide polymorphisms (π) are commonly used to measure the genetic diversity of species or populations (Hao et al., 2014). The 19 nematode population isolates used in this study had a total of 14 haplotypes with an Hd of 0.942, indicating high haplotype diversity in *M enterolobii* populations in Guangdong Province. In contrast, the total nucleotide diversity was very low (π = 0.00429). The high haplotype diversity and low nucleotide diversity suggests that a bottleneck of *M enterolobii* populations occurred, followed by rapid population expansion. The accumulation of mutations could have led to the high haplotype diversity observed among these populations, which maintained a high nucleotide similarity. High haplotype diversity and low nucleotide diversity is very common in invertebrates with large maternal effective populations and strong reproductive abilities (Grant & Bowen, 1998; Lavery et al., 2008). Tajima's D and Fu's FS analyses confirmed that the all *M enterolobii* populations population might have undergone a population expansion event during evolution.

From the phylogenetic results (Fig. 1), the 19 *M enterolobii* populations (Clade A and Clade B) in this study and the sequence selected on NCBI (Clade C) are not on the same branch. The *M enterolobii* populations in this study were all isolated from the mulberry root, other *M enterolobii* were parasitic on *Ipomoea aquatica, Zingiber officinale, Daucus carota, Psidium guajava*, and came from different regions. The 19 *M enterolobii* populations in this study were clustered on the same branch. The *M enterolobii* selected on NCBI gathered on another branch. It further shows that these 19 *M enterolobii* populations have no geographical barriers and had sufficient genetic exchanges. At the same time, it also shows that the genetic differentiation of *M enterolobii* from different hosts is different. However, whether *M enterolobii* will have a biased active host selection, and whether *M enterolobii* of the same host will have genetic changes due to infecting different host plants, this will require further research. From the evolutionary perspective of 19 *M enterolobii* populations in a single cluster, most of the *M enterolobii* populations are clustered in Clade A, and the genetic relationship is inconsistent with their geographical distribution. It can be seen that the *M enterolobii* populations parasitizing on mulberry may have slowly evolved from a population in a certain area.

In this study, we evaluated the genetic diversity of *M enterolobii* from different regions using mitochondrial genes. Rashidifard (2019) performed a molecular characterisation of *M enterolobii* populations from South Africa and found that mitochondrial genes could accurately reflect the phylogenetic relationship of different *M enterolobii* populations. Tigano M et al. (2010) analysed the genetic diversity

of different *M enterolobii* isolates using intergenic regions (IGS) of the rDNA, *COII*, and *16S* rRNA; they found a low level of diversity among the isolates tested, suggesting that *M enterolobii* is a genetically homogeneous root-knot nematode. However, in this study, we found evidence of extensive gene exchange among different populations. These discrepancies could be explained by the fact that, in contrast to previous studies, the host of the *M enterolobii* isolates used in this study was mulberry. The second instar larvae and the male had limited mobility because the *M enterolobii* colonized mainly in the host roots. Therefore, we hypothesized that the egg and larvae remaining in the soil after the formation of the egg were separated from the plant root. They will be the result of long-distance passive propagation of human activities such as agricultural operations or wind, rain and water currents. Additionally, all the nematode populations used in this study were isolated from different regions of the south of China, and geographical factors may have caused nucleotide polymorphisms. The use of different genes and research methodologies are also possible causes of discrepancies.

Deng et al. (2016) analysed the genetic diversity of *Rotylenchulus reniformis* from different geographic regions in China based on the sequence of *COII*; they found a high variation in *COII-LrRNA* sequence, suggesting the high genetic variation among different *Rotylenchulus reniformis* populations, contributing to their ability to adapt to environmental changes. Consistent with our findings, Newton et al. (2003) used random amplification of polymorphic DNA (RAPD) markers to analyse the genetic diversity of soybean cyst nematodes and found that phylogenetic clusters were not associated with geographic proximity. Consistently, we found that the phylogenetic relationship of different *M enterolobii* populations was not related to the geographic location of the isolates.

The haplotype analysis revealed that the haplotype Hap9 is shared among mulberry root-knot nematode species in Guangdong Province and that the other haplotypes have evolved from Hap9. Furthermore, the low genetic variation among the populations and the absence of a relationship between haplotype and the geographical region further support that the different mulberry root-knot nematode populations isolated from Guangdong may have originated from the same population (Yu, 2009). The topological structure of the phylogenetic tree also suggested that the absence of phylogenetic relationship among the different *M enterolobii* populations from Guangdong Province. The haplotype Hap9 diverged into three large groups, while no clustering was observed between different haplotypes from the same geographical region; these findings further support the genetic flow among different mulberry root-knot nematodes in Guangdong Province, leading to a low genetic diversity. It is likely that during the genetic flow, genes involved in drug resistance are also transferred, enabling the transmission of mulberry root-knot nematodes.

Declarations

DATA AVAILABILITY STATEMENT

The assembled gene sequences of M. enterolobii are available in NCBI (MN907170-MN9071718, respectively). The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

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Contributions

Conceptualization: Hudie Shao, Chunping You and Yan Feng; Methodology: Chunping You and Hudie Shao; Formal analysis and investigation: Hudie Shao and Pan Zhang; Writing-original draft preparation: Hudie Shao; Writing-review and editing: Chuanren Li, Chunping You, Hudie Shao and Pan Zhang; Funding acquisition: Chunping You.

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Ethics declarations

Conflict of Interest

The authors declare no conflict of interest, neither financial, commercial nor personal, that could be construed to influence the work reported in this paper.

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Tables

Table1. *Meloidogyne enterolobii* samples from mulberry garden in china used in this study.

Group	City	town	Sample number	Variety	Acquisition time	number of root samples (Female/ Article)
YZ	Guangzhou	Baiyun	GB	Yuexiu63	2016.7; 2017.8	10
	Huadu	Baosang	HB	Japanese mulberry	2016.5; 2017.10	10
	Guangzhou	Huanong	GH	Shi 4	2018.4	10
	Yunfo	Luoding	YL	Tang10×109	2017.9	10
	Foshan	Shede	FS	Tang10	2017.8	10
	Guangzhou	Nansha	GN	Sijiguo sang	2018.5	10
	Guangzhou	Panyu	GP	Da10	2018.5	10
YB	Yingde	Dawan	YD	Da10	2016.8; 2017.5	10
	Shaoguang	Shensuo	SS	Yuesang11	2017.6	10
	Qingyuan	Daqiao	QD	Kangqing10	2017.9	10
	Qingyuan	Qingcheng	QQ	283×Kangqing 10	2018.6	10
	Qingyuan	Yangshan	QY	Sha er×109	2017.6	10
	HunanProvince Changsha	Linmen	HCL	32×109	2016.3; 2017.9	10
YN	Zhangjiang	Nanchang	ZN	230	2017.10	10
	Maoming	Mingsheng	MM	Kang10	2017.10	10
	Zhangjiang	Shuixi	ZS	Kang 10×230	2017.10	10
	Kai ping	Baihe	KB	Kangqing 10 hao	2016.11	10
	Zhangjiang	Siyuan	ZS1	Kangqing 10 hao	2017.10	10
	Maoming	Huazhou	MH	Xiang7920	2017.9	10

Note: YB: Northern Region of Guangdong Province, China

YN: Southern Region of Guangdong Province, China

YZ: Central Region of Guangdong Province, China

Table 2 Accession numbers of 19 *M enterolobii* populations based on *mtCOII* gene

Sample number	GenBank number
GB	MN907170
HB	MN907171
GH	MN907172
YL	MN907173
FS	MN907174
GN	MN907175
GP	MN907176
YD	MN907177
QD	MN907178
QQ	MN907179
SS	MN9071710
QY	MN9071711
MH	MN9071712
ZS	MN9071713
ZN	MN9071714
MM	MN9071715
KB	MN9071716
ZS1	MN9071717
HCL	MN9071718

Table 3. Haplotypes identified in *mtCOII* gene in *M enterolobii* populations

Haplotype	Haplotype sequence	N	No.	Geographic distribution	Haploty pefrequency
Hap1	CTCGAACTGAGTGCT	1	MN907171	HB	5.26%
Hap2	CTCTAACTCAGTCCT	1	MN907178	QD	5.26%
Hap3	CTCTAGCGCAAGAGT	1	MN907177	YD	5.26%
Hap4	CTCTAATTCAAGACT	1	MN9071713	ZS	5.26%
Hap5	CTGGAAGTCAATACT	1	MN907174	FS	5.26%
Hap6	CTCCAACTCAAGACT	1	MN9071718	HCL	5.26%
Hap7	CTCTAACTCGATACT	1	MN907172	GH	5.26%
Hap8	CTCTAACTCAATACT	5	MN907173,MN9071715,MN907179,MN9071710,MN9071716	YL,MM,QQ, SS,KP	26.36%
Hap9	CTCTAACTCCATACT	1	MN9071714	ZN	5.26%
Hap10	CTGTGACTCAATGCT	1	MN907175	GN	5.26%
Hap11	CTCGAAGTCGGTGCT	1	MN907170	GB	5.26%
Hap12	CTGGAAGTCGATACT	1	MN907176	GP	5.26%
Hap13	TCCTAACTCAATACT	2	MN9071712,MN9071717	MH,ZS1	10.52%
Hap14	CTCTAACTCAATCCG	1	MN9071711	QY	5.26%

Table 4 Genetic variation analyses of 19 *M enterolobii* geography groups based on *mtCOII* gene

Source of variation	d.f	Sum of squares	Variance components	Percentage of Variation
Between groups	2	1.035	0.00938 Va	2.00
Within group	16	7.333	0.45833 Vb	98.00
Total variation	18	8.368	0.46771	

Table 5 Haplotype diversity of *mtCOII* gene and nucleotide polymorphism level in each group

group	Hap number	Hd	π	K	Tajima's D	Fu's Fs
YB	4	0.900	0.00361	3.00	-0.74682	-0.3.534
YZ	7	0.867	0.00209	1.733	1.64797	-0.761
YN	4	1.000	0.00470	3.905	1.02876	-3.646

Table 6 Genetic differentiation and gene flow analysis of *M enterolobii* groups based on *mtCOII* gene

Group 1	Group 1	Nm	Fst	Kxy
YZ	YB	4.5	0.10237	4.17143
YZ	YN	1.94	0.20353	4.04762
YB	YN	2.088	0.19318	2.93333

Nm, pairwise comparisons based on gene flow; Fst, genetic variance within the subpopulation relative to the total genetic variance; Kxy, Average number of nucleotide differences

Figures

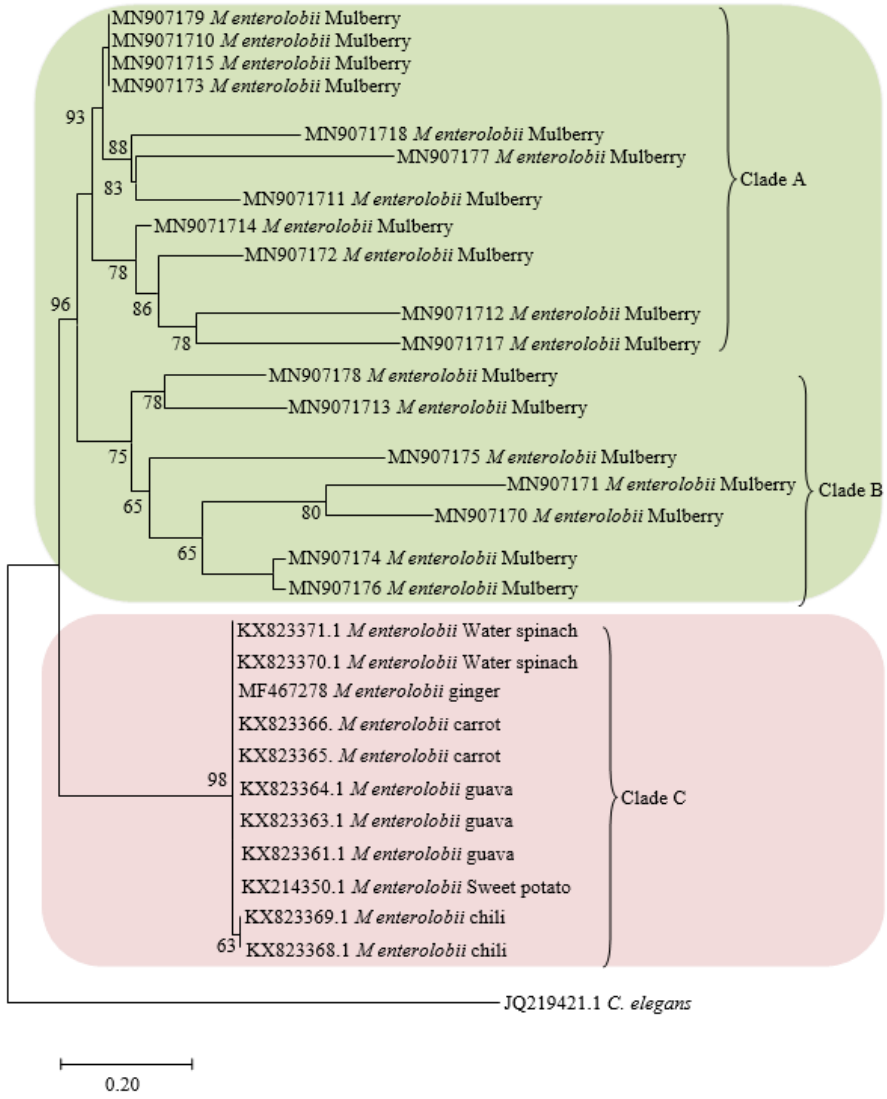

Figure 1

Phylogenetic trees showing relationship between 19 M. enterolobii populations and other M. enterolobii available on GenBank based on mtCOII gene. C. elegans were used as out-group. The trees were constructed using MEGA7.0 by neighbour-joining method with bootstrap replications of 1,000. Frequency values higher than 50% were displayed on the trees. The sequences of 19 M. enterolobii are indicated in shadow green. The sequences available on GenBank are shaded in purple.

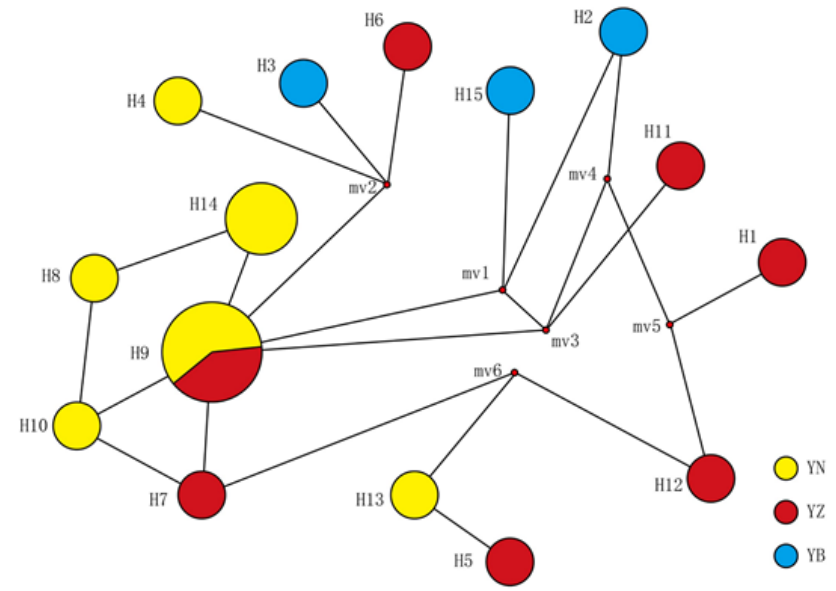

Figure 2

Median-Joining haplotype network of 19 M enterolobii populations based on mtCOII gene using NETWORK 5.0 soft. The links are labeled by the nucleotide positions to designate transitions. Cycle size is roughly proportional to the haplotype frequency, the groups are indicated by different colors.

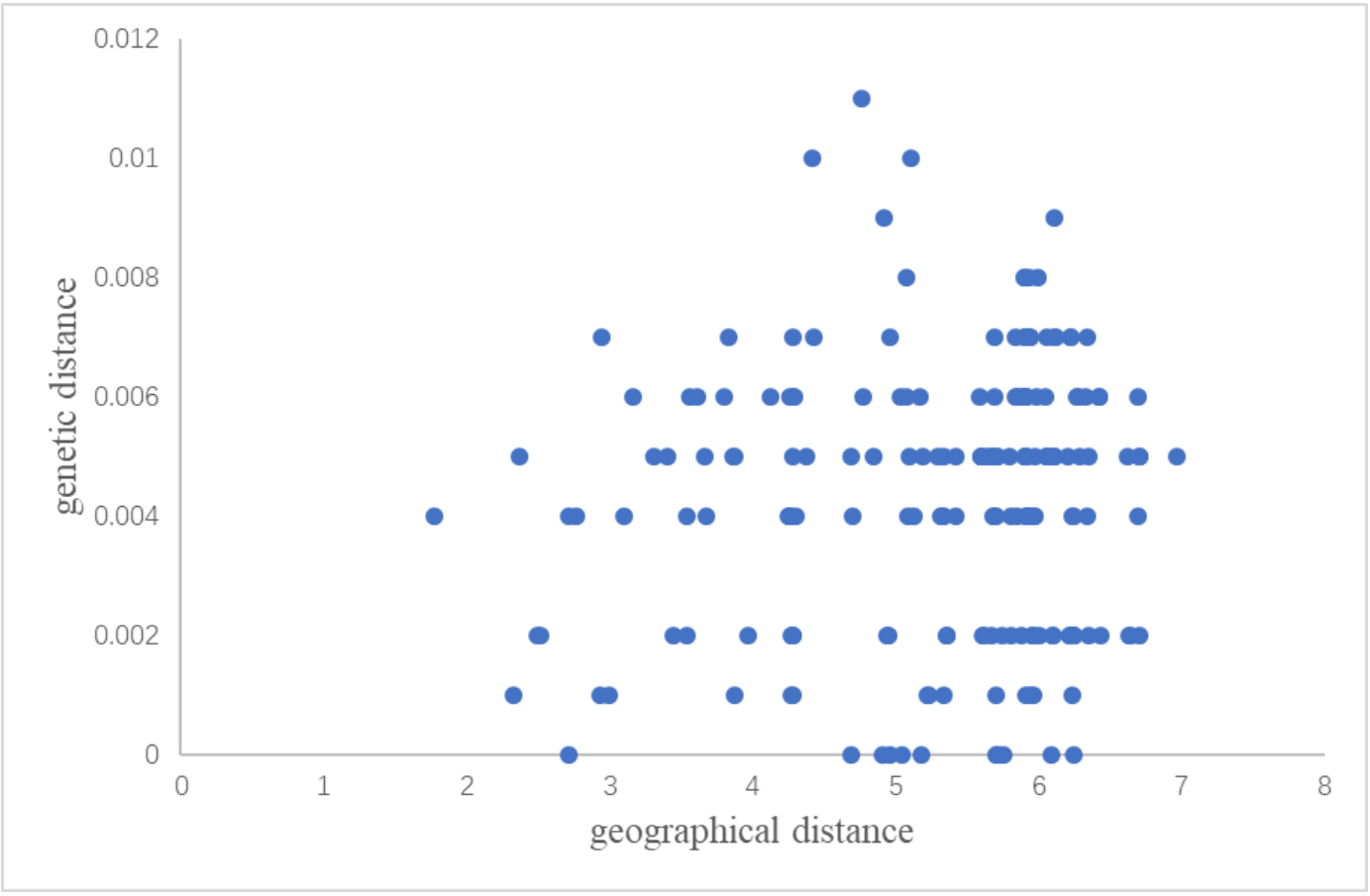

Figure 3

Correlation between genetic distance(Appendix 1) and geographical distance(Appendix 2) in the 19 M enterolobii populations based on mtCOII gene using SPSS soft. Relationship between genetic and geographic distance matrices for M enterolobii (r=0.093, p=|-0.204|>0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Appendix.docx