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Gene Sequence Variation and Genetic Differentiation of Mitochondrial COI in Genus *Saperda* (Coleoptera:Cerambycidae:Lamiinae)

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Abstract

In this study，the total DNA of nine species of *Saperda* (*Lopezcolonia*) *octopunctata* (Scopoli, 1772), *Saperda* (*Lopezcolonia*) *scalaris* (Linnaeus, 1758), *Saperda interrupta* Gebler, *Saperda Alberti* (Plavilstshikov), *Saperda* (*Saperda*) *similis* Laicharting, 1784, *Saperda* (*Compsidia*) *populnea* (Linnaeus, 1758),*Saperda* (*Saperda*) *carcharias* (Linnaeus, 1758), *Saperda* (*Lopezcolonia*) *perforata* Pallas, 1773 and *Saperda ohbayashi* were extracted . Two partial sequences of mitochondrial gene and one partial sequence of nuclear gene were amplified. Comparing the COI sequence with the DNA barcode data in GenBank can

effectively identify the related species of *Saperda*. It will be applied to the rapid identification of some species of *Saperda* in imported wood at ports, and improve the detection rate of plant quarantine.

Keywords: Genus *Saperda*;mtDNA;COI;detection;sequence analysis

Saperda Fabricius, 1775, belongs to Insecta, Coleoptera, Cerambycidae, Lamiinae. There are more than 70 species of *Saperda* in the world and 23 species in China. For many years, many species of *Saperda* pests were often found in imported wood at ports. From October 2013 to October 2015, dozens of quarantine pests of *Saperda* (non Chinese species) were intercepted at ports. It is an urgent task to exclude the Chinese species of *Saperda* and intercept the quarantine pests of *Saperda* (non Chinese species). It is difficult and inaccurate for port inspection and quarantine personnel to exclude Chinese species of *Saperda* only from the external morphology, which often needs expert identification and review. The study on the genetic differentiation and phylogeny of *Saperda* population can provide scientific basis for the study on the relationship between the source of *Saperda*, the occurrence mechanism and the accurate quarantine and identification of *Saperda*. It also has important theoretical and practical significance for improving the level of forecasting and monitoring technology of *Saperda* and formulating sustainable population control strategy.

Saperda (*Lopezcolonia*) *octopunctata* (Scopoli, 1772) is a non Chinese Quarantine Pest prohibited entry by the state. It was first intercepted from Tilia saw blade imported from Romania at Hainan port in 2009. *Saperda ohbayashi* is an experimental specimen from Japan, but not distributed in China. It has been found in northern China that *Saperda* (*Lopezcolonia*) *scalaris* (Linnaeus, 1758)、*Saperda interrupta* Gebler、*Saperda alberti* (Plavilstshikov)、*Saperda* (*Saperda*) *similis* Laicharting, 1784、*Saperda* (*Compsidia*) *populnea* (Linnaeus, 1758)、*Saperda* (*Saperda*) *carcharias* (Linnaeus, 1758)、*Saperda* (*Lopezcolonia*) *perforata* Pallas, 1773 have been distributed. In this paper, the total DNA of these nine species of *Saperda* were extracted and the partial sequences of two mitochondrial genes and one nuclear gene were amplified. The COI sequences were compared with the DNA barcodes in GenBank to identify different species of *Saperda*.

1 Materials and Methods

1.1 Materials

Nine species specimens of *Saperda* (*Lopezcolonia*) *octopunctata* (Scopoli, 1772)、*Saperda* (*Lopezcolonia*) *scalaris* (Linnaeus, 1758)、*Saperda interrupta* Gebler、*Saperda alberti* (Plavilstshikov)、*Saperda* (*Saperda*) *similis* Laicharting, 1784、*Saperda* (*Compsidia*) *populnea* (Linnaeus, 1758)、*Saperda* (*Saperda*) *carcharias* (Linnaeus, 1758)、*Saperda* (*Lopezcolonia*) *perforata* Pallas, 1773 and *Saperda ohbayashi* were bought or presented from the cerambycids research experts who all collected the fresh specimens in 2015.If the samples received are not immersed in absolute ethanol, they should be immediately immersed in absolute ethanol and stored in - 80 ℃ refrigerator. The specimen information is shown in Table 1:

Table 1 Source information of *Saperda* specimens

Name of specimen	Host	Collecting place and time	Collector	Corresponding sequencing number
Saperda octopunctata Scopoli	*Tilia*	Czech Republic ,2015	J. Borucky	9
Saperda alberti Plavilstshikov	*Populus nigra* var. *italia*	Saima Town, Fengcheng, Dandong, Liaoning, China	Zang Kai	8
Saperda ohbayashii (Podany,1963)	Absent	2015-VI-14 Japan	Yamanashi	7
Saperda interrupta (Gepler,1825)	Absent	2015-VI-11 Japan	Yamanashi	6

Saperda similis Laicharting	Larval rearing	2012-V-10	MORAVIA exl Bruntal-Razova V. Sima	5
Saperda populnea（Linnaeus）	Absent	BOHEMIA Tatenice 2015-VI-11Czech Republic ,2015		4
Saperda perforate Pallas	Larval rearing	BOHEMIA Czech Republic ,2015		3
Saperda scalaris（Linnaeus）	Larval rearing	BOHEMIA Czech Republic ,2015	Lanskrounenv	2
Saperda carcharias（Linnaeus）	*Salix caprea*	2015-V-18Czech Republic ,2015	Vladimir Sima	1

1.2 Methods

1.2.1 Design and synthesis of primers

The universal primers designed by Simon et al. (1994) were used to amplify COI gene,C1-J-2183 （ 5′- CAACATTTATTTTGATTTTTGG -3′ ） and TL2-N-3014 （ 5′-TCCAATGCACTAATCTGCCATATTA -3′）.

1.2.2 Extraction of genomic DNA

In this experiment, the leg or chest muscles of *Saperda* adult were used as experimental materials, and the phenol chloroform isoamyl alcohol method was used to extract DNA. The specific experimental steps were modified according to the extraction method of Sambrook et al. (1989).

1.2.3 PCR amplification and sequencing

PCR reaction system: 10 × PCR buffer (containing Mg2 +) 2.5 μ L, 2.5mmol/l dNTP 2 μ L, Taq DNA polymerase (5U) 0.2 μ L, C1-J-2183/ TL2-N-3014（10 μmol/L） 1 μ L, DNA template 1 μ L, water supplement to 25 μ L. The PCR reaction conditions were as follows: pre denaturation at 95 ℃ for 5 min, followed by 35 cycles: denaturation at 95 ℃ for 30 sec,

annealing at 47 ℃ for 40 sec, extension at 72 ℃ for 1 min, extension at 72 ℃ for 5 min, and heat preservation at 4 ℃. The amplified products were detected by 1% agarose gel electrophoresis and purified to Shenzhen Huagi Gene Technology Service Co., Ltd.

1.2.4 Sequence analysis

BioEdit v7.0.5, GeneDoC, Clustal W and MEGA 5.10 software were used to compare and analyze the sequences, and the phylogenetic tree was constructed.

2 Results and Analysis

2.1 Detection of PCR products

By agarose gel electrophoresis, 9 species of *Saperda* were amplified with clear and uniform bands (Fig. 2.1), and negative control and blank control did not amplify strips. The results showed that the COI gene primer C1-J-2183/ TL2-N-3014 could be used to amplify the gene fragments of 9 species of Cerambycidae, and the amplified fragments were consistent with the target fragments.

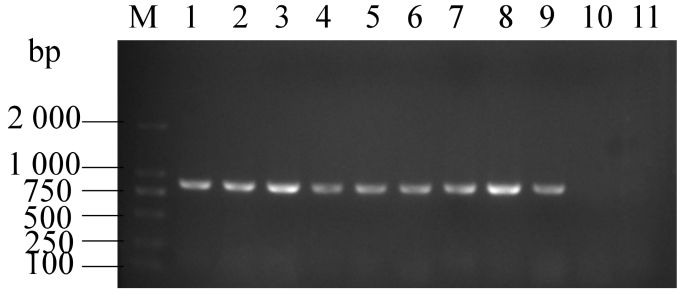

Fig 1 Agarose gel electrophoresis of PCR products amplified

2.2 Sequence composition and variation of different species of *Saperda*

The results showed that the average length of mtDNA CO I gene sequences of 9 different species of *Saperda* was 729 bases, the average contents of A, T, C and G were 30.9%, 38.1%, 16.1% and 14.9% respectively, the average contents of A + T and G + C were 69.0% and 31.0% respectively, and the average contents of A + T were more than 2 times of the average contents of G + C; the average contents of A + T at codon 1 were 62.6% and the average contents of A + T at codon 2 were 62.6% The average content of C and G in locus 2 was 2.0% and 10.2%, respectively. The average content of a was the highest, reaching 44.1%, and

the average content of T was 43.7%. The results showed that the frequency of base usage of codon was obviously biased. The contents of mtDNA co I a, t, C and G in different species of *Saperda* are different, as shown in the following table (Table 2). The results showed that there were significant genetic differences in base content among different species of *Saperda*.

Table 2 Comparison of base composition of COI gene in mtDNA of 9 species of *Saperda*

Species of *Saperda*	Sequence length/bp	base content /%			
		T	A	C	G
Saperda octopunctata	729	38.4	31.8	15.5	14.3
Saperda alberti	729	37.3	32.4	15.5	14.8
Saperda ohbayashii	729	38.4	28.5	17.3	15.8
Saperda interrupta	729	40.0	28.3	16.3	15.4
Saperda similis	729	38.3	31.3	16.0	14.4
Saperda populnea	729	36.8	31.8	17.0	14.4
Saperda perforate Pallas	729	37.7	31.3	15.5	15.5
Saperda scalaris	729	37.6	31.3	16.0	15.1
Saperda carcharias	729	38.1	31.3	16.2	14.4

There were 476 conserved sites and 253 variant sites in sequence length of 729 bp , with a variation rate of 34.71%. There were 171 parsimony informative loci and 82 self descendant loci, accounting for 32.41% of the total. The composition of gene base substitution of mtDNA CO I of nine different species of *Saperda* is shown in Table 3. Generally, the conversion between sequences is equivalent to transversion. Statistics show that the nucleotide substitution at different codon sites is different, especially at codon 2, the frequency of conversion and transversion is high. The conversion accounts for 61.82% of the total conversion, and the transversion accounts for 87.93% of the total transversion. It is a synonymous substitution, which causes less substitution of amino acids. The results show that the transversion mainly occurs between A and T, and there is almost no transversion between G and C; the transformation mainly occurs between T and C; the average ratio of transformation and transversion is 1.0. The frequency of transition and transversion of codon 1 is almost the same, and the frequency of transition of codon 3 is much higher than that of transversion.

Table 3 base substitution composition of mtDNA CO I gene in 9 species of *Saperda*

codon	ii	si	sv	*R*	Identical Pairs				Transitional Pairs		Transversional Pairs				Total
					TT	CC	AA	GG	TC/CT	AG/GA	TA/AT	TG/GT	CA/AC	CG/GC	
1	238	2	2	1.1	104	52	46	37	1/1	0/0	0/0	0/0	0/0	0/0	243
2	158	34	51	0.7	72	6	80	0	13/14	3/3	21/19	1/1	4/5	0/0	243
3	220	19	5	3.9	58	33	66	63	7/6	3/3	2/1	0/0	0/1	0/0	243
Average	615	55	58	1.0	234	91	191	100	21/21	7/7	23/20	1/1	5/6	1/1	729

Notes：ii（Identical Pairs）；si（Transitional Pairs）；sv（Transversional Pairs）；*R*=Transitional Pairs/Transversional Pairs

2.3 Analysis of genetic distance among different species of *Saperda*

Based on the Kinura-2-Parameter model, the genetic distances among different species of *Saperda* were analyzed (Table 4). The average genetic distance of the nine species was 0.271, while that of *Saperda similis* and *Saperda carcharias* was the smallest, only 0.001. The results showed that the genetic distance between *Saperda interrupta* and *Saperda alberti* was the largest (0.927), the genetic distance between *Saperda interrupta* and *Saperda populnea* was the second largest (0.909), and the genetic distance between *Saperda octopunctata* and *Saperda interrupta* and that between *Saperda octopunctata* and *Saperda ohbayashii* was 0.631 and 0.594 respectively,which were a little larger .

Table 4 Genetic distance of mtDNA COI gene sequences of 9 species of *Saperda*

Species of *Saperda*	9	8	7	6	5	4	3	2	1
9									
8	0.066								
7	0.594	0.731							
6	0.631	0.927	0.141						

5	0.023	0.095	0.399	0.484				
4	0.182	0.111	0.565	0.909	0.128			
3	0.084	0.067	0.410	0.556	0.066	0.171		
2	0.071	0.060	0.372	0.560	0.034	0.086	0.018	
1	0.032	0.092	0.390	0.501	0.001	0.104	0.067	0.029

Notes：1.*Saperda carcharias*；2.*Saperda scalaris*；3.*Saperda perforata*；4.*Saperda populnea*；5.*Saperda similis*；6.*Saperda interrupta*；7.*Saperda ohbayashi*；8.*Saperda alberti*；9.*Saperda octopunctata*

2.4 Construction of phylogenetic analysis

One sequence of 9 species of longicorn was selected and analyzed by mega4.0 software. The neighbor joining method (N-J method), maximum parsimony method (M-P method) and UPGMA method were used to draw the diagrams respectively. The confidence of each branch was tested by "bootstrap" 1000 times. The obtained tree diagrams are respectively Fig. 2, Fig. 3 and Fig. 4. From the topological structure, the results of the three clustering methods are basically consistent. The results showed that nine species of *Saperda* were clustered into one branch, and the same species were all independent, and the self expanding support rate was 100%. The similarity between the two species *Saperda similis* and *Saperda carcharias* was the highest, which was aggregated with 100% confidence, indicating that the two species were closely related. In the phylogenetic tree, *Saperda scalaris* and *Saperda interrupta* were clustered into one branch with high confidence, indicating that they were closely related. There are obvious differences among different species of *Saperda*, indicating that this sequence can be used as the basis for molecular identification of *Saperda*.

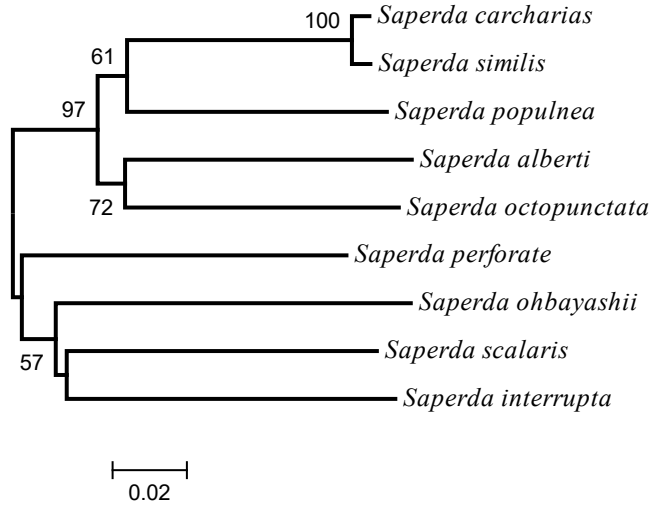

Fig 2 N-J tree based on the analysis of COI gene sequence of the 9 *Saperda* species

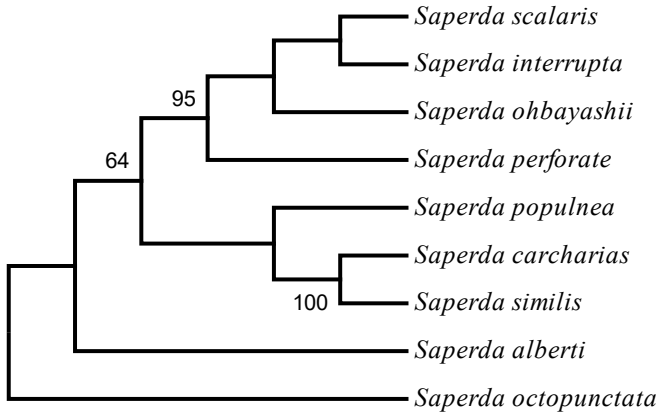

Fig 3 M-P tree based on the analysis of COI gene sequence of the 9 *Saperda* species

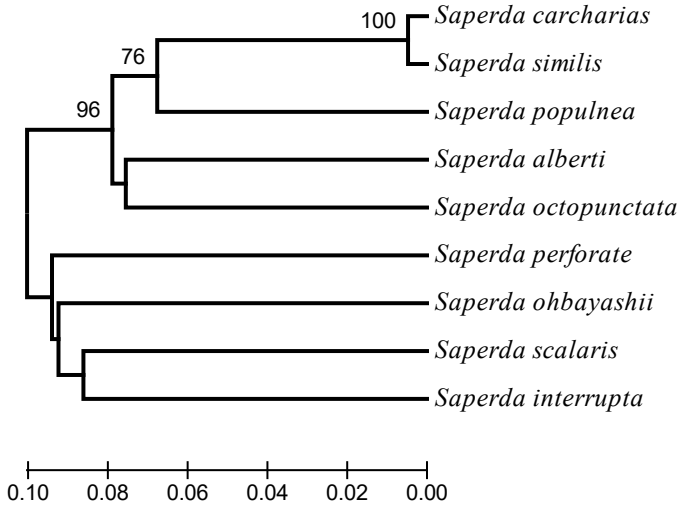

Fig 4 UPGMA tree based on the analysis of COI gene sequence of the 9 *Saperda* species

3 Discussion

3.1 DNA barcoding can be used to identify the species of *Saperda*

DNA barcoding technology needs two conditions to identify species: first, each species has a unique DNA barcode and there is no shared sequence among different species; second, the genetic distance between species should be much greater than that within species. In this study, the COI sequences of 9 species of *Saperda* were amplified by universal primers, and a fragment of 729bp was obtained.The results show that the genetic distance between species was significantly greater than that within species.

There was no overlap between intraspecific genetic distance and interspecific genetic distance, and interspecific genetic distance was significantly greater than intraspecific genetic distance.Based on the DNA barcode sequence, the phylogenetic tree was constructed by using the adjacency method and the maximum parsimony method. The results showed that the same species clustered into a small branch, and the node support rate was 100%. The results of genetic distance and phylogenetic tree showed that the fragment could be used as the basis for the analysis and identification of Cerambycidae species.

3.2 Validation of classification results using molecular barcode data

There are many species of *Saperda* with a long history of classification. Due to intraspecific variation, such as color and stripe variation, it is often difficult to accurately and quickly identify the species of *Saperda*. The results of NJ tree and MP tree showed that a species of

Saperda was an independent species at the molecular level.

Due to the limitation of specimen, only 9 species of *Saperda* were studied in this study. There are still many problems to be clarified in the classical taxonomy of *Saperda* based on external morphology and male genitalia. The external morphological characteristics of some species are very similar. For example, the color and spot position of *Saperda octopunctata*, *Saperda punctata*, *Saperda perforate* Pallas and *Saperda alberti* Plavilstshikov species are easy to confuse. In the future, molecular barcode data should be supplemented on the basis of morphological classification and accurate identification, and NCBI and bold database should be used for large-scale research in order to identify and analyze the phylogenetic relationship of Cerambycidae more quickly and accurately.

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References

[1] Li Jing, An Shiheng, Yin Xinming. Phylogeny of 12 species of Lamiinae beetles based on COI gene sequence. Central China insect research (Vol. 7), 2011:243

[2] Li Jing, An Shiheng, Zhang Hongfei, et al. Characteristics and phylogeny of mitochondrial Co II gene in some species of Lamiinae beetles. Journal of Henan Agricultural University, 2013, 47 (6): 715-721

[3] Ma Jian, Li Chengde, Deng Liwen, et al. Assisted identification of several poplar stem borer larvae based on COI gene restriction endonuclease. Journal of Northeast Forestry University, 2010, 38 (5): 83-87

[4] Zheng Sizhu, Zhang Kai, Yang Xiaojun, et al. Comparison of COI genes and preliminary systematic study

[5] on Monochamus. Acta Entomologica Sinica, 2015, 52 (2): 370-381

[6] Zhang Shuang, Su Xiaoyu, Huang Dazhuang, et al. Sequence variation and genetic differentiation of mitochondrial COI gene in different geographic populations of Germari cerambycids. Sericultural Science, 2015, 41 (2): 234-238

[7] Kethidi D R, Roden D B, Ladd T R, et al. Development of SCAR markers for the DNA-based detection of the Asian long-horned beetle, Anoplophora glabripennis (Motschulsky). Arch Insect Bioehem Physiol, 2003, 52(4): 193-204