Advanced Materials Research Vols. 183-185 (2011) pp 757-767
Online available since 2011/Jan/20 at www.scientific.net

doi:10.4028/www.scientific.net/AMR.183-185.757

Molecular phylogenetic relationships of different color forms within *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) based on sequences of 12S rRNA and 16S rRNA gene

Dabin Yao [1, a], Defu Chi [1, b], Qingyu Wu [1, c], Xiaocan Li [1, d], Jia Yu [1, e]

[1] Laboratory of Insect Molecular and Biology, Life Science Institute of Northeast Forestry University, Harbin 150040, China

[a]email:yaodabin840226@163.com,[b]email:chidefu@126.com,
[c]email:594756227@qq.com,[d]email:lxcfgz@163.com,[e]email:rainbest@yahoo.com.cn

Keywords: *Harmonia axyridis* Pallas, 12S rRNA, 16S rRNA, molecular phylogeny

Abstract: The high level phylogenetic relationships within *Harmonia. axyridis* Pallas (Coleoptera: Coccinellidae) was investigated based on nucleotide data from two molecular markers (12S rRNA, 16S rRNA), along with those of four species obtained from GenBank, including *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, *Propylea quattuordecimpunctata* as outgroup taxa to construct NJ (Neighbor Joining), MP (Maximum Parsimony) and Bayesian trees using Mega 4.0, PAUP (Version 4.0 b10) and Mrbayes (version 3.1.2) software packages. Nucleotides composition, pairwise distances, ratio of transition / transversion, considerable variable sites of those segments were analyzed. The results from phylogenetic trees indicated that *H .axyridis* var. *spectabilis* Fald. and *H. axyridis* ab. *lunata* Hem. had very close relationships. *H. axyrisdis* ab.½123456789-*undevigintisignata* Fald. and *H. axyridis* ab. *succinea* Hope. were closely related. *H. axyridis* var. *axyridis* Pallas. was an original group, and it had derived to be a independent forma earlier than the other 11 formas within *H. axyridis* according to the molecular phylogeny analyzing results. Previous hypotheses on the evolution of different color forms of *H. axyridis* Pallas based on morphological data was supported by our molecular systematic analyzing results.

Introduction

The Asian lady beetle, Harmonia axyridis Pallas (Coleoptera: Coccinellidae) belongs to *Harmonia or Leis* genus [1], mainly distributes in Asia, with a wide geographic distribution, such as in China, Mongolia, Korea, Japan and so on. *H. axyridis* Pallas is important predator, feeding on aphids, mealybugs, coccids, psyllids and other pests [2, 3, 4, 5, 6]. Therefore, *H. axyridis* Pallas was widely released to control aphids and some other pests [7, 8]. However, the broadly distribution and plenty color forms of *H. axyridis* Pallas have brought difficulties on their identification or nomination. Meanwhile, we know little about phylogenetic relationships within *H. axyridis* Pallas. With the development of molecular phylogeny, the traditional research methods show their shortcomings in resolving systematics problems. Therefore, molecular phylogeny is becoming more and more popular to resolve systematics problems [9, 10].

In recent years, molecular methods based on mitochondrial genes have been widely used to investigate the relationships within species [11], and the relationships of closely related species [12] for their rapid evolution rate. More recently, the usage of mitochondrial genes to study the

phylogenetic relationships and evolutionary history of certain groups has been advocated [13, 14, 15, 16, 17]. The aim of this study was to explore the relationships within different color forms of *H. axyridis* Pallas. We examined partial sequences of 12S rRNA and 16S rRNA gene of 12 different color forms of *H. axyridis* Pallas, analyzed nucleotides composition, pairwise genetic distances, ratio of transition / transversion, considerable variable sites, using NJ, MP and Bayesian methods to construct phylogenetic trees, preliminarily explored the relationships of 12 different color forms of *H. axyridis* Pallas.

Materials and methods

Taxon sampling

H. axyridis Pallas were collected in September 2007, from the Maoershan Forestry Centre of Northeast Forestry University, Harbin, Heilongjiang Province, China. Different color forms of *H. axyridis* Pallas analyzed in this study were listed in Table 1. Those Asian lady beetles were flash-frozen in liquid nitrogen after being collected and then stored at -40°C until being used. Whenever possible, three or more individuals from each specimen were sampled. A single individual from each forma was used for DNA extraction. Generic classifications used in this study were based largely on Yuan Rongcai's classfication [18]. The information data of outgroups, including *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, *Propylea quattuordecimpunctata* were acquired from GenBank as shown in Table 2.

Table 1 List of investigated taxa with GenBank accession

Code	Scientific name(by Yuan Rongcai)	Genbank accession number	
		16S rRNA	12S rRNA
1	*H. axyridis* ab. *succinea* Hope.	FJ601208	FJ769241
2	*H. axyridis* ab.1347-*octosignata* Yuan.	FJ601202	FJ769242
3	*H. axyridis* ab.12345678-*sedecimsignata* Mls.	FJ601203	FJ769243
4	*H. axyridis* ab.123456789-*duodevigintisignata*(Mls.)Yuan.	FJ601205	FJ769244
5	*H. axyrisdis* ab.½123456789-*undevigintisignata* Fald.	FJ601206	FJ769245
6	*H.axyridis* ab. *transverifascia* Bar.	FJ601213	FJ769246
7	*H. axyridis* ab. *conspicua* Fald.	FJ601212	FJ769247
8	*H. axyridis* ab. *spectabilis* Fald.	FJ601207	FJ769248
9	*H. axyridis* ab. *falcata* Hem.	FJ601209	FJ769249
10	*H. axyridis* ab. *lunata* Hem.	FJ601210	FJ769250
11	*H. axyridis* ab. *circumscripta* Hem.	FJ601204	FJ769251
12	*H. axyridis* var. *axyridis* Pallas.	FJ601211	FJ769252

Table 2 GenBank sequences data used for this study

Code	Species	Locality	Genbank accession number	
			16S rRNA	12S rRNA
13	*Adalia bipunctata*	United Kingdom: Cambridgeshire	AM779599	AM779607
14	*Calvia quatuordecimguttata*	United Kingdom: Cambridgeshire	AM779600	AM779612
15	*Coccinella septempunctata*	United Kingdom: Cambridgeshire	AM779602	AM779608
16	*Propylea quattuordecimpunctata*	United Kingdom: Cambridgeshire	AM779605	AM779610

DNA extraction, PCR amplification and sequencing

Total genomic DNA samples were extracted from single individuals using the improved CTAB-method of Yao Dabin [19]. All DNA samples were stored at -20°C. Portions from two

mitochondrial ribosomal genes (12S rRNA and 16S rRNA) were amplified. The primers used to amplify the two fragments are shown in Table 3. The 12S rRNA and the16S rRNA gene segments were amplified via PCR in ABI 9700 Thermal Cycler. Both 12S rRNA and 16S rRNA were proven to be useful in *H. axyridis* Pallas molecular systematics.

Table 3 Primers used for amplification and sequencing of *H. axyridis* Pallas taxa

Locus	Primer Name	Sequence (5'-3')	Source
12S rRNA	12S-F	TACTATGTTACGACTTAT	Kambhampati [20]
	12S-R	AAACTAGGATTAGATACCC	Kambhampati [20]
16S rRNA	16S-F	CCGGTCTGAACTCAGATCACGT	Bouchon *et al*. [21]
	16S-R	CGCCTGTTTAACAAAAACAT	Bouchon *et al*. [21]

PCR reactions were performed in a total volume of 20μL. Reactions contained 10×reaction buffer (including 100mmol/L Tris-HCl pH 8.3, 30 mmol/L $MgCl_2$, 500 mmol/L KCl)2μL, 10mmol/L dNTPs 1μL, 10μmol/L of each primer 1μL, ddH_2O 13.8μL, 1.0U of Taq DNA polymerase, DNA template 1μL(containing DNA 20-50ng). Amplifications were performed in a thermocycler with an initial denaturation step of 94°C for 4 min, then 33 cycles of 94°C for 1 min, 40-54°C for 1 min, 72°C for 2 min, and a final extension step at 72°C for 10 min. Reactions were then cooled to 4°C until removal. Annealing temperature for each forma was optimized and listed in Table 4. PCR products were checked on a 1.2% agarose gel, and successfully amplified fragments were purified either directly with BioDev Gel Extraction System B according to manufacturer's instructions, or through a second electrophoresis on 1.2% agarose gel, with excision of the PCR product from the gel and subsequently purified with Min Elute kits. Purified DNA was cycle-sequenced in both forward and reverse directions using the ABI Big Dye® Terminator v.3.1 cycle sequencing kit and was carried out in an ABI 3730 Capillary Electrophoresis Genetic analyser at Jinsite Biotechnology (Nanjing, China). Sequence electropherograms were edited manually by comparing both strands using BioEdit v.3.0.9 [22]. All sequences were deposited in GenBank shown in Table 1.

Table 4 The best annealing temperature for PCR amplifications

Code	Specimen	16S rRNA	12S rRNA
1	*H. axyridis* ab. *succinea* Hope.	54°C	54°C
2	*H. axyridis* ab. 1347-*octosignata* Yuan.	46°C	46°C
3	*H. axyridis* ab.12345678-*sedecimsignata* Mls.	46°C	47.5°C
4	*H. axyridis* ab.123456789-*duodevigintisignata* (Mls.)Yuan.	54°C	54°C
5	*H. axyrisdis* ab.½123456789-*undevigintisignata* Fald.	54°C	54°C
6	*H. axyridis* ab. *transverifascia* Bar.	46°C	47.5°C
7	*H. axyridis* ab. *conspicua* Fald.	54°C	55°C
8	*H. axyridis* ab. *spectabilis* Fald.	54°C	54°C
9	*H. axyridis* ab. *falcata* Hem.	54°C	54°C
10	*H. axyridis* ab. *lunata* Hem.	54°C	54°C
11	*H. axyridis* ab. *circumscripta* Hem.	46°C	46°C
12	*H. axyridis* var. *axyridis* Pallas.	40°C	40°C

Phylogenetic analysis

Alignments for the individual gene data matrices were generated using similarity calculated method with Clustal X version 1.81 software package [23]. Sequences were adjusted manually and no indels (insertions/deletions) were required for the alignments. Prior to phylogenetic reconstruction, nucleotide compositions, pairwise genetic distances, ratio of transition / transversion, and considerable variable sites were analyzed for the two data sets (12S rRNA and 16S rRNA gene sequences).

Neighbor Joining analysis

Mega 4.0 software package was used for the Neighbor Joining (NJ) analyses for each gene region [24]. Distance matrixes were constructed from the aligned sequences choosing Kimura 2-parameter model, and all characters were treated as unordered, and gaps were treated as missing data. The estimates of nodal support on distance trees were derived using bootstrap analysis with 1000 replications.

Maximum parsimony analysis

Data for each gene region was analyzed under the criterion of maximum parsimony (MP) using PAUP 4.0b10 [25]. For all MP analyses, heuristic tree searches were conducted with the tree bisection and reconnection (TBR) option, and 1000 random-taxon-addition replicates held 50 trees per replicate, and treated sequence indels as missing data. Estimates of nodal support were computed with bootstrap (1000 standard replicates).

Bayesian analysis

Bayesian phylogenetic inference was used to estimate the tree topology. We used MrBayes 3.1.2 software package to estimate the posterior probability distribution [26, 27]. The gamma distribution of rate variation across sites was approximated by a discrete distribution with four rate categories, each category being represented by its mean rate. All chains, including coupled chains in the same run, were started from different, randomly chosen trees. Searches were run for one million generations with four chains (one cold and three heated), sampling every 100 generations. Convergence was assessed by the standard deviation of split frequencies of the two independent MrBayes runs, by the convergence diagnostic for individual parameters employing potential scale reduction factor (PSRF), and by the achievement of stationarity of the log likelihood values of the cold chain. The initial 1000 sampled trees were discarded as ‘‘burn-in”, and the remainder used to construct a 50% majority rule consensus tree. The GTR + I + G model was chosen for the two partitions of the concatenated data set (12S rRNA and 16S rRNA) for Bayesian analyses as a means to deal with the heterogeneity within the data [28].

Results

General properties of sequences

We sequenced a 349-394 bp piece of 12S rRNA (12S), a 511-537 bp piece of 16S rRNA (16S), and the alignment of 12S and 16S regions resulted in aligned data matrices of 372bp and 525bp respectively, 897bp in total. Results of the separate analyses were very sensitive to weighting variations. The nucleotide compositions of the two data sequences are similar, and have the high A + T content both in 12S and 16S sequences as shown in Table 5. The result revealed a bias in favor to thymine and adenine bases in mitochondrial markers (12S and 16S), as currently observed in insects [29, 30, 31], and it is also called the A+T-rich region. Of the two gene regions, 12S rRNA had the higher proportion of variable sites (205bp; 52%), and parsimony informative sites (21bp; 5.3%). The 16S region was 13.4% (74bp) variable, with 7.8% (43bp) of these sites being parsimony informative. The ratio of transition/transversion obtained among 12 different color forms of *H. axyridis* Pallas based on 12S and 16S characters was 0.7378, 0.5450, respectively.

Table 5 Base composition of molecular markers

	Bases composition (%)					
	A	C	G	T	A+T	C+G
12S rRNA	41.6	12.8	8.8	36.8	78.4	21.6
16S rRNA	38.7	14.4	10.7	36.2	74.9	25.1

Uncorrected pairwise genetic distances among different color forms of *H. axyridis* Pallas shown on Table 6, were calculated by Mega 4.0 based on 12S and 16S characters used in phylogenetic analysis. Average genetic pairwise distances of different color forms of *H. axyridis* Pallas were 0.0513 and 0.0640 respectively. From the pairwise genetic distances based on 12S and 16S sequences, we could easily find that *H. axyridis* var. *spectabilis* Fald. and *H. axyridis* ab. *lunata* Hem. had the shortest genetic distances which was 0.0102 only. The genetic distances between *H. axyridis* ab. *succinea* Hope. and *H. axyrisdis* ab.½123456789-*undevigintisignata* Fald. was 0.0271, which was very short also. These results indicated that they had very close blood relationships. *H. axyridis* ab. *succinea* Hope. and *H. axyridis* var. *axyridis* Pallas. had the farthest relationship as the distance is the longest (0.0975 for 12S, 0.0738 for 16S).

Table 6 Pairwise genetic distances among ingruop taxa

code	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0.0129	0.0259	0.0422	0.0233	0.0259	0.0154	0.0529	0.0287	0.0154	0.0504	0.0975
2	0.0658	0	0.0286	0.0422	0.0207	0.0286	0.0181	0.0448	0.0287	0.0530	0.0476	0.0954
3	0.0292	0.0685	0	0.0393	0.0259	0.0476	0.0286	0.0530	0.0311	0.0338	0.0451	0.0924
4	0.0650	0.0458	0.0656	0	0.0421	0.0393	0.0422	0.0449	0.0128	0.0475	0.0287	0.0825
5	0.0271	0.0687	0.0313	0.0648	0	0.0259	0.0233	0.0447	0.0286	0.0207	0.0447	0.0927
6	0.0700	0.0706	0.0706	0.0646	0.0708	0	0.0286	0.0530	0.0311	0.0338	0.0451	0.0924
7	0.0666	0.0727	0.0667	0.0679	0.0669	0.0683	0	0.0420	0.0286	0.0421	0.0367	0.0952
8	0.0687	0.0710	0.0692	0.0679	0.0685	0.0683	0.0556	0	0.0476	0.0102	0.0259	0.0954
9	0.0671	0.0717	0.0667	0.0689	0.0673	0.0675	0.0458	0.0548	0	0.0339	0.0313	0.0847
10	0.0550	0.0675	0.0560	0.0668	0.0563	0.0681	0.0688	0.0692	0.0688	0	0.0529	0.0961
11	0.0689	0.0708	0.0683	0.0560	0.0683	0.0654	0.0654	0.0565	0.0658	0.0654	0	0.0915
12	0.0738	0.0692	0.0673	0.0675	0.0673	0.0658	0.0689	0.0654	0.0688	0.0681	0.0660	0

Note. Above diagonal: pairwise genetic distances based on 12S rRNA, below diagonal: pairwise genetic distances based on 16S rRNA. The codes of Table 6 were consistent with those of Table 1.

Molecular phylogenetic analysis

Analyses according to 12S and 16S sequences separately

Results of NJ and MP analyses were shown in Fig.1-Fig.4, using data matrices of 372bp and 525bp aligned from 12S and 16S sequences respectively. The topological structures of the NJ and MP trees were essentially similar, different in the bootstrap values of some clades and positions of some correlated branches. For example, *H. axyridis* ab. *conspicua* Fald. and *H. axyridis* ab. *transverifascia* Bar. gathered in one branch in 16S NJ tree (Fig.2) and 16S MP tree (Fig.4), whereas did not gather together in 12S NJ tree (Fig.1) and 12S MP tree (Fig.3). Even so, NJ and MP analyses based on 12S and 16S data drew the same conclusion that *H. axyridis* var. *spectabilis* Fald. and *H. axyridis* ab. *lunata* Hem., *H. axyrisdis* ab.½123456789-*undevigintisignata* Fald. and *H. axyridis* ab. *succinea* Hope. had very close relationships, with very strong bootstrap support, which conformed to the morphology classification results. *H. axyridis* var. *axyridis* Pallas. separated a clade earlier than the other 11 different color forms of *H. axyridis* Pallas, which implied *H. axyridis* var. *axyridis* Pallas. was a original group, and this result also consistent with morphology characters.

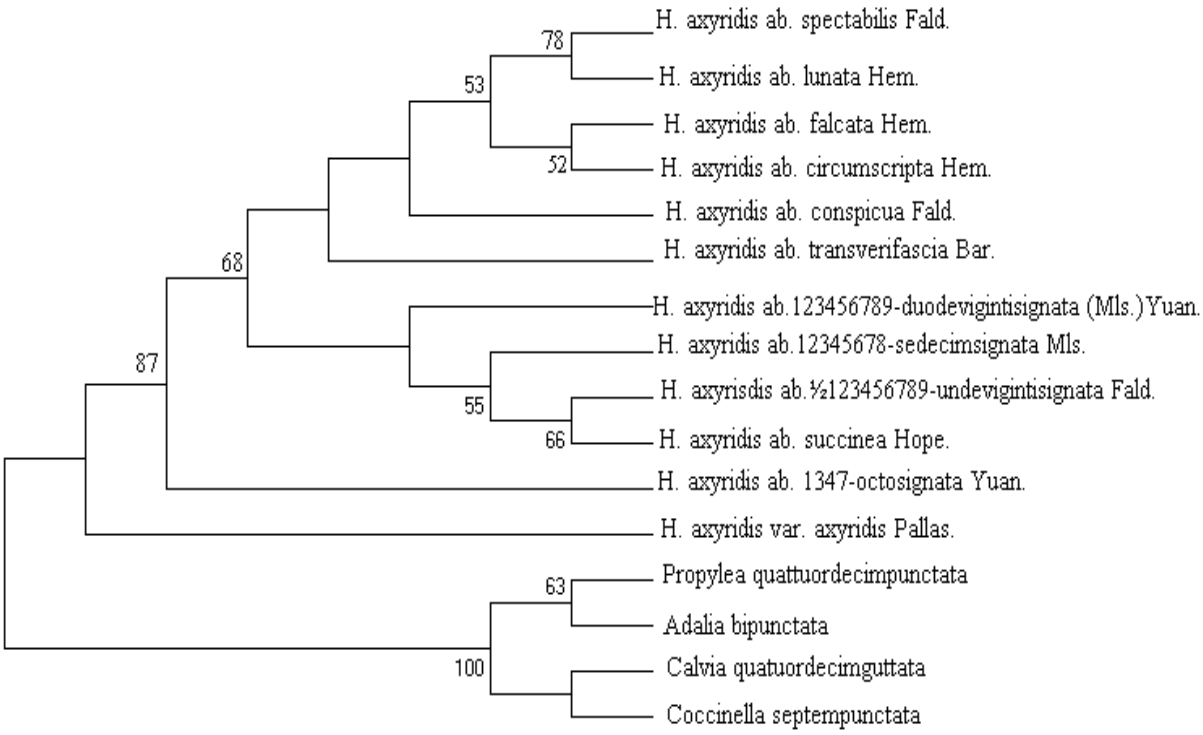

Fig. 1 NJ tree amongst different color forms of *H. axyridis* Pallas based on partial sequences of 12S rRNA (12S) gene.

Note: The bootstrap confidence values of each branch are shown above the branch (values>50 shown). *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, and *Propylea quattuordecimpunctata* were used as outgroups.

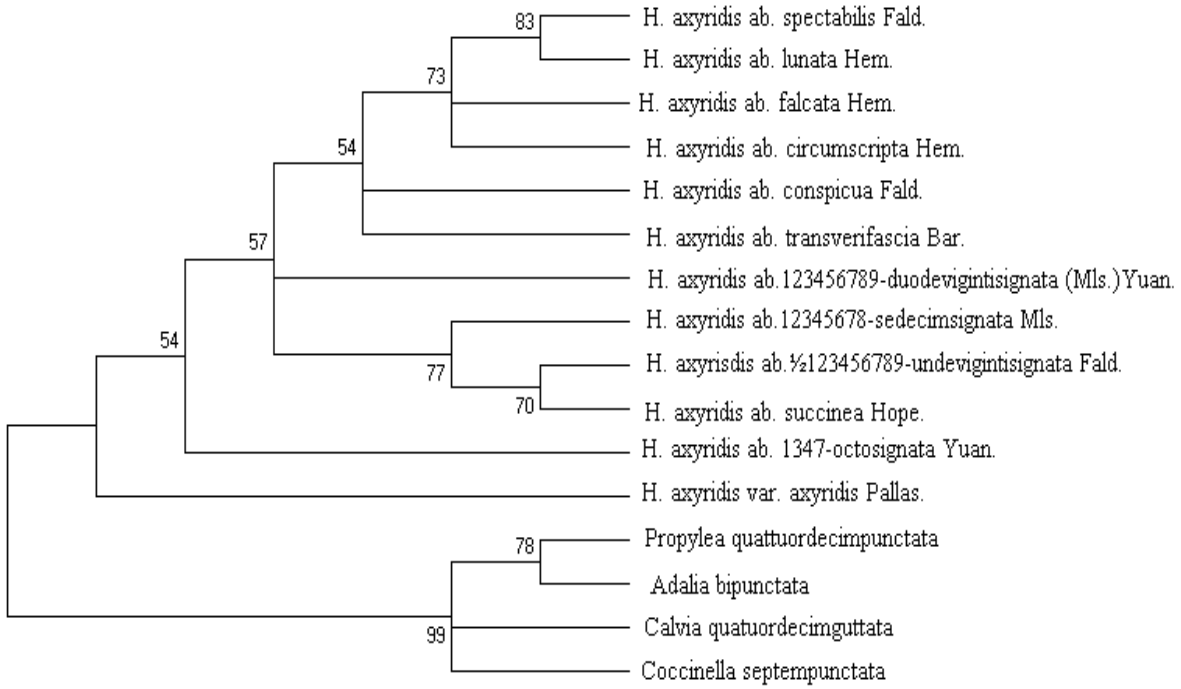

Fig. 2 NJ tree amongst different color forms of *H. axyridis* Pallas based on partial sequences of 16S rRNA (16S) gene.

Note: The bootstrap confidence values of each branch are shown above the branch (values>50 shown). *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, and *Propylea quattuordecimpunctata* were used as outgroups.

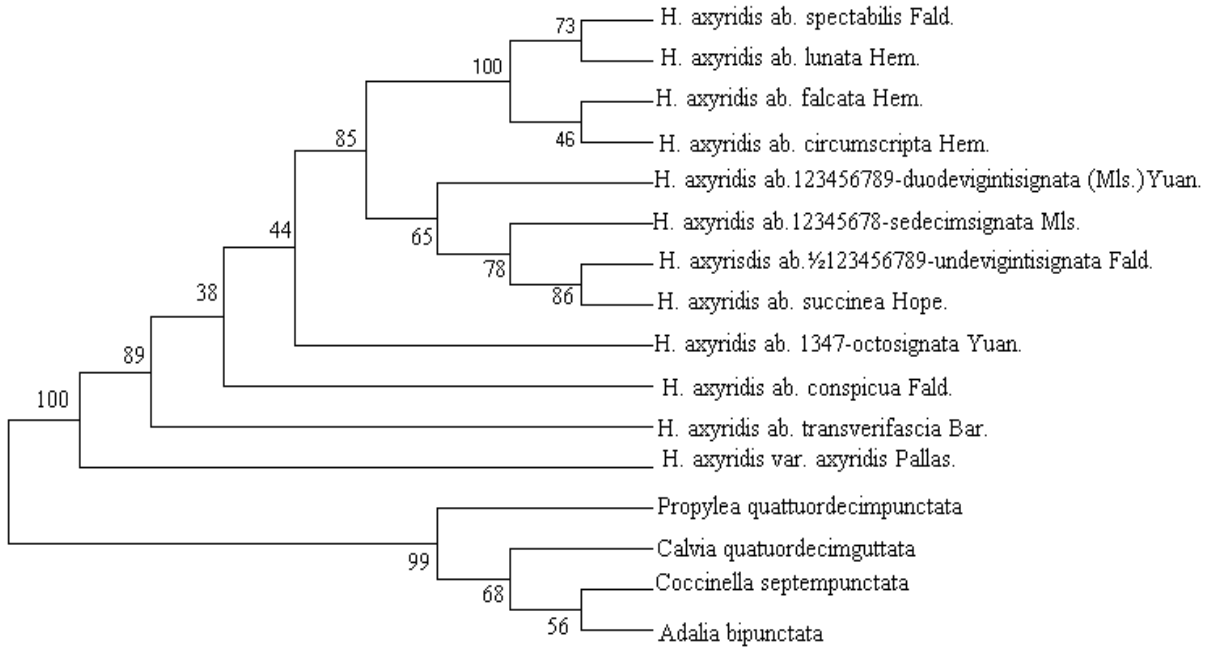

Fig. 3 MP tree from 12S rRNA (12S) data set among different color forms of *H. axyridis* Pallas.

Note: The bootstrap confidence values of each branch are shown above the branch. *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, and *Propylea quattuordecimpunctata* were used as outgroups.

(1863 steps; CI = 0.5204; RI = 0.5100)

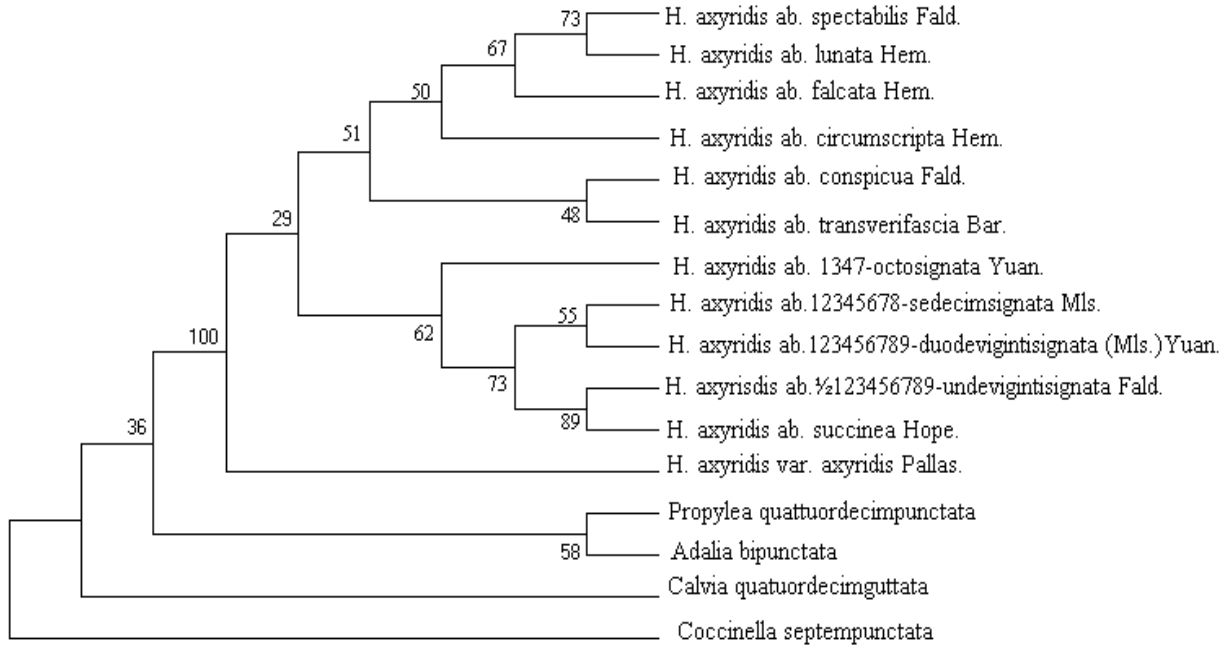

Fig. 4 MP tree from the data set of 16S rRNA (16S) gene among different color forms of *H. axyridis* Pallas.

Note: The bootstrap confidence values of each branch are shown above the branch. *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, and *Propylea quattuordecimpunctata* were used as outgroups. (1927 steps; CI = 0.5379; RI = 0.6573)

Analyses according to combined 12S and 16S sequences information

Bayesian phylogenetic inference was used to estimate the combined data. These analyses have been performed for aligned data matrices of 897bp in total, considering all 16 sampled taxa, covered by 12S+16S molecular data set. The 50% majority rule consensus trees (Fig.5) obtained

from the two partitions of the concatenated data set (12S+16S) were largely consistent in the separate analyses. From the Bayesian tree (Fig.5), the species referred here could be divided into three groups. Only *H. axyridis* var. *axyridis* Pallas. gathered a clade earlier than all the other 11 different color forms of *H. axyridis* Pallas. This result indicated that *H. axyridis* var. *axyridis* Pallas. was an original group. The second clade was comprised of *H. axyridis* ab. *transverifascia* Bar., *H. axyridis* ab. *conspicua* Fald., *H. axyridis* ab. *circumscripta* Hem., *H. axyridis* ab. *falcata* Hem., *H. axyridis* ab. *lunata* Hem. and *H. axyridis* ab. *spectabilis* Fald.. In this clade, *H. axyridis* ab. *lunata* Hem. was sister to *H. axyridis* ab. *spectabilis* Fald. with very strong support value. The third clade was consisted of five color forms of *H. axyridis* Pallas, which were *H. axyridis* ab. *succinea* Hope., *H. axyridis* ab. 1347-*octosignata* Yuan., *H. axyridis* ab.12345678-*sedecimsignata* Mls., *H. axyridis* ab.123456789-*duodevigintisignata* (Mls.)Yuan. and *H. axyrisdis* ab. ½123456789-*undevigintisignata* Fald.. In this clade, *H. axyridis* ab. *succinea* Hope. was sister to *H. axyrisdis* ab. ½123456789-*undevigintisignata* Fald. with very strong support value.

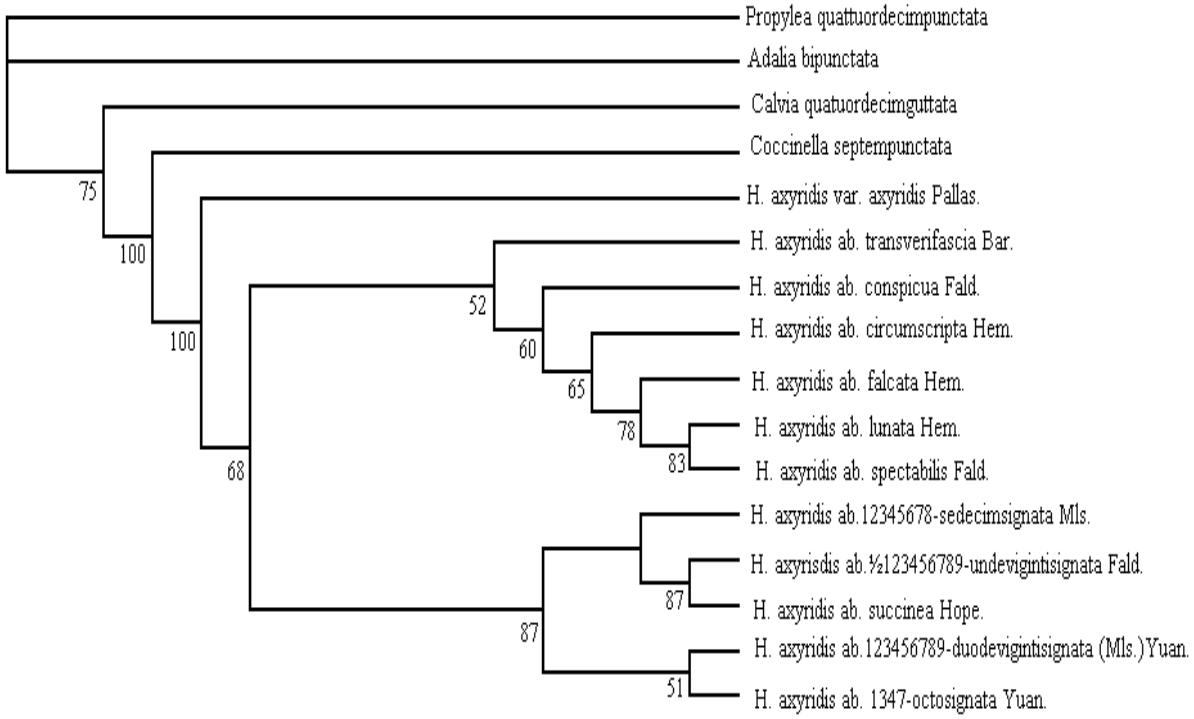

Fig.5 Majority-rule consensus tree resulting from the Bayesian inference of the combined data set (12S+16S)

Note: The bootstrap confidence values of each branch are shown above the branch (values>50 shown). *Adalia bipunctata*, *Calvia quatuordecimguttata*, *Coccinella septempunctata*, and *Propylea quattuordecimpunctata* were used as outgroups.

Conclusion

This study analyzed phylogenetic relationships within *H. axyridis* Pallas. of 12 different color forms based on two molecular markers (12S, 16S). Nucleotides composition, pairwise distances, ratio of transition / transversion, considerable variable sites of those segments were analyzed. Both separated and combined results indicated that *H .axyridis* var. *spectabilis* Fald. and *H. axyridis* ab. *lunata* Hem., *H. axyrisdis* ab.½123456789-*undevigintisignata* Fald. and *H. axyridis* ab. *succinea* Hope. both had very close relationships, and *H. axyridis* var. *axyridis* Pallas. was an original group, and had an earlier clade comparing with the other 11 different color forms of *H. axyridis* Pallas according to the research on molecular systematics. Previous hypotheses on the evolution of different color forms of *H. axyridis* Pallas based on morphological data are briefly tested, and supported, by our new data set.

Discussion

Although the molecular tool has been commonly used for phylogenetic purposes since more than 20 years, the present study is the first phylogenetic reconstruction for a high-level clade within *H. axyridis* Pallas using molecular data, and it is actually the first analysis that combines two molecular markers (12S and 16S) in a phylogenetic framework for this insect. Our results are consequently significant not only for the model clade we study, but also more generally for *H. axyridis* Pallas as a whole.

There has been much discussion about "the best evolutionary rate" for a gene to be suitable for phylogeny reconstruction [32]. Phylogeneticists used the reverse argument to discern particular events in the history from the pattern of diversification [33]. However, the usefulness of a particular DNA sequence also depends on the tempo and mode of genealogical differentiation of the organisms carrying them. Recently, mitochondrial genes have been widely used to investigate the relationships within species and the relationships of closely related species for their rapid evolution rate [11, 12], and mitochondrial genes have almost universally A+T biased in insects [35, 36, 37, 38, 39].

The phylogenetic information carried by 12S and 16S appears both differently and widely distributed on the trees we constructed. The results that the three methods (NJ, MP and Bayesian) used in this study are not always the same. That's because different method has different algorithm. NJ method depends upon distance algorithm, two sequences evolution distance decides the cladogram topological structure, and the branch length represents its evolution distance. MP method optimizes the cladogram using the least separate steps to explain difference of multiple aligned sequences. For close kinship sequences, using the MP method to construct phylogenetic tree would be better, because the supposition used is the least. Bayesian inference is a based on Bayesian model cluster algorithm. Overall, topologies obtained in this study with MP analyses were very similar to the Bayesian inference results. Because the Bayesian tree is used to show nodal support values obtained using bootstrap proportions of parsimony and maximum likelihood (ML), as well as Bayesian posterior probabilities. As expected, Bayesian posterior probabilities were higher or equal to bootstrap proportions found with NJ and with MP.

Acknowledgements

We are grateful to the insect molecular laboratory of Northeast Forestry University to collect samples of different types of *H. axyridis* Pallas. This material is based upon work supported by the National Natural Science Foundation of China under grant (30671694).

References

[1] Liu Chongle: Chinese Economy Entomography, science press publishers, p. 59-60,(1963)
[2] Jiang Yongchen and Zhu Peiyao: A research summary of *Harmonia axyridis* Pallas, Jiangxi Plant Protection, Vol. 16(1), p. 30-34, (1993)
[3] Brown MW and Miller SS: Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*, Entomological News, Vol. 109, p. 143-151, (1998)
[4] Li Yan, Shao Yangguang, Sun Hui:. Simple and convenient experimental materials of genetics, *Harmonia axyridis* Pallas, *Bio. bll*., Vol. 36 (3), p.12-15, (2001)
[5] Wang Xiaoyi and Shen Zuorui: Progress of applied research on multicolored Asian ladybird beetle, Entomological Knowledge, Vol. 39, p.255-261,(2002)
[6] Brown MW: Role of aphid predator guild in controlling spirea aphid populations on apple in

West Virginia, U.S.A. Biological Control, Vol.29, p.189-198, (2004)
[7] Hodek I and Honek A: Ecology of coccinellidae, Kluwer academic publishers, The Netherlands, p.464,(1996)
[8] Seo MJ and Youn YN: The Asian ladybird, *Harmonia axyridis*, as biological control agents: Predacious behavior and feeding ability, Kor. J. Appl. Entomol, Vol.39, p.59-71, (2000)
[9] Zakharov EV, Caterino MS, Sperling FAH: Molecular phylogeny, historical biogeography, and divergence time estimates for swallowtail butterflies of the genus *Papilio* (Lepidoptera: Papilionidae), Syst. Biology, Vol. 53(2), p.193-215, (2004)
[10] Kawamura Y: A report on the public symposium Part I "Recent advances in zoogeography" at the annual meeting of the Mammalogical Society of Japan: Recent progress in paleontological studies on the Quaternary mammals of Japan, Mammalian science, Vol. 47, p.107-114,(2007)
[11] Avise JC: Phylogeographyraphy: the history and formation of species, Cambridge: Harvard University Press, (2000)
[12] Sperling F: Butterflies: Evolution and EcologyTaking Flight. Butterfly species and molecular phylogenies, Chicago: University of Chicago Press, p. 431-458, (2003)
[13] Wahlberg N and Zimmermann M: Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera: Nymphalidae) inferred from mitochondrial DNA sequences, Cladistics, Vol.16, p. 347-363, (2000)
[14] Carole DG., Vincent L, Catherine H: A molecular phylogeny of anseriformes based on mitochondrial DNA analysis, Molecular Phylogenetics and Evolution, Vol.23, p. 339-356, (2002)
[15] Kankare M and Shaw MR: Molecular phylogeny of *Cotesia* Cameron, 1891 (Insecta: Hymenoptera: Braconidae: Microgastrinae) parasitoids associated with Melitaeini butterflies(Insecta: Lepidoptera: Nymphalidae: Melitaeini), Molecular Phylogenetics and Evolution, Vol.32, p.207-220, (2004)
[16] Murray D and Prowell DP: Molecular phylogenetics and evolutionary history of the neotropical Satyrine Subtribe Euptychiina (Nymphalidae: Satyrinae), Molecular Phylogenetic and Evolution, Vol.34, p.67-80, (2005)
[17] Liu Ruoyu, Yang G.ongshe, Lei Chuzhao: The genetic diversity of mtDNA D-loop and the origin of chinese goats, Acta Genetica Sinica, Vol.33, p. 420-428, (2006)
[18] Yuan Rongcai, *et al*.. Investigation and research about *Harmonia axyridis* from changbai mountain, Jilin Agricul.Sci., Vol.4 (1), p.45-54,(1994)
[19] Yao, Dabin, Chi Defu, Yu Jia, Li Xiaocan: An Improved Method for Extraction of Nucleic Acid from Coleopteran Insects, Journal of Northeast Forestry University, Vol.37(1), p.86-88,(2009)
[20] Kambhampati S: A Phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes, Proe.Natl.Acad.Sci., U.S.A., Vol.92, p.2017-2020,(1995)
[21] Bouchon D, Souty-Grosset C, Raimond R.: Mitochondrial DNA variation and markers of species identity in two penaeid shrimp species: Penaeus monodon Fabricius and *P. japonicus* Bate, Aquaculture, p.127, (1994)
[22] Hall TA: BioEdit: a user-friendly biological sequence alignment editor and analysis, Program for Windows 95/98/NT.Nucleic Acids Symposium Series, Vol.41, p.95-98,(1999)
[23] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin Fand Higgins DG.: The Clustalx windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Res., Vol.24, p. 4876-4882,(1997)
[24] Tamura K, Dudley J, Nei M, Kumar S: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0.Molecular Biology and Evolution, Vol.24, p.1596-1599, (2007)
[25] Swofford DL: PAUP: Phylogenetic Analysis Using Pasimony (and other methods) Version 4.0b10, Sunderland, Massachusetts: Sinauer Associates, (2002)
[26] Ronquist F and Huelsenbeck JP: MRBAYES 3: Bayesian phylogenetic inference under mixed models, Bioinformatics, Vol.19, p.1572-1574,(2003)
[27] Huelsenbeck JP and Ronquist F: MrBayes: Bayesian inference of phylogeny. Bioinformatics, Vol.17, p.754-755,(2005)

[28] Zwickl DJ: Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, University Texas, Austin, (Computer program distributed by the author at: http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html) (2006)
[29] Brown WM: The mitochondrial genome of animals. New York, p.95-130,(1985)
[30] Ding Fangmei and Huang Yuan: Molecular evolution and phylogenetic analysis of some species of Oedipodidae (Orthoptera : Caelifera) in China based on complete mitochondrial ND2 gene, Acta Entomologica Sinica, Vol.51, p.55 -601,(2008)
[31] Liu Yan. and Huang Yuan: Sequencing and analysis of complete mitochondrial genome of *Chorthippus chinensis* Tarb. Chinese Journal of Biochemistry and Molecular Biology, Vol.24, p.329-335, (2008)
[32] Yang Ziheng: On the best evolutionary rate for phylogenetic analysis. Systematic Biolog, Vol.47, p. 125-133,(1998)
[33] Pagel M: Inferring evolutionary processes from phylogenies. Zoologica Scripta, Vol.26,p.331-348,(1998)
[34] Fauron CMR and Wolstenholme DR: Structural heterogeneity of mitochondrial DNA molecules within the genus *Drosophila*, Proc. Natl. Acad. Sci., U.S.A., Vol.73, p.3623-3627,(1976)
[35] Van Raay TJ and Crease TJ: Partial mitochondrial DNA sequnce of the crustacean *Daphnia pulex*, Curr. Genet., Vol. 25, p.66-72, (1994)
[36] Zhang Dexing and Hewitt GM: Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies, Biochem. Syst. Ecol., Vol.25, p.99-120,(1997)
[37] Wirth T, Le Guellec R, Veuille M:Directional substitution and evolution of nucleotide content in the Cytochrome Oxidase II gene in Earwigs (Sermapteran Insects), Molecular Phylogenetic and Evolution, Vol.16, p.1645-1653,(1999)
[38] Yamauchi MM, Miya MU, Nishida M: Complete mitochondrial DNA sequence of the Japanese spiny lobster, *Panulirus japonica* (Crustacea:Decapoda),Gene, Vol.295, p.89-96,(2002)
[39] Kilpert F, Podsiadlowski L: The complete mitochondrial genome of the sea slater, *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region features, BMC Genomics, Vol.7, p.241,(2006)

Environmental Biotechnology and Materials Engineering
10.4028/www.scientific.net/AMR.183-185

Molecular Phylogenetic Relationships of Different Color Forms within Harmonia axyridis Pallas (Coleoptera: Coccinellidae) Based on Sequences of 12S rRNA and 16S rRNA Gene

10.4028/www.scientific.net/AMR.183-185.757

DOI References

[6] Brown MW: Role of aphid predator guild in controlling spirea aphid populations on apple in West Virginia, U.S.A. Biological Control, Vol.29, p.189-198, (2004) doi:10.1016/S1049-9644(03)00153-1

[9] Zakharov EV, Caterino MS, Sperling FAH: Molecular phylogeny, historical biogeography, and divergence time estimates for swallowtail butterflies of the genus Papilio (Lepidoptera: Papilionidae), Syst. Biology, Vol. 53(2), p.193-215, (2004) doi:10.1080/10635150490423403

[13] Wahlberg N and Zimmermann M: Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera: Nymphalidae) inferred from mitochondrial DNA sequences, Cladistics, Vol.16, p. 347-363, (2000) doi:10.1006/clad.2000.0136

[16] Murray D and Prowell DP: Molecular phylogenetics and evolutionary history of the neotropical atyrine Subtribe Euptychiina (Nymphalidae: Satyrinae), Molecular Phylogenetic and Evolution, ol.34, p.67-80, (2005) doi:10.1016/j.ympev.2004.08.014

[17] Liu Ruoyu, Yang G.ongshe, Lei Chuzhao: The genetic diversity of mtDNA D-loop and the origin of chinese goats, Acta Genetica Sinica, Vol.33, p. 420-428, (2006) doi:10.1016/S0379-4172(06)60069-3

[21] Bouchon D, Souty-Grosset C, Raimond R.: Mitochondrial DNA variation and markers of species identity in two penaeid shrimp species: Penaeus monodon Fabricius and P. japonicus Bate, Aquaculture, p.127, (1994) doi:10.1016/0044-8486(94)90420-0

[24] Tamura K, Dudley J, Nei M, Kumar S: MEGA4: Molecular Evolutionary Genetics Analysis MEGA) software version 4.0.Molecular Biology and Evolution, Vol.24, p.1596-1599, (2007)

doi:10.1093/molbev/msm092

[35] Van Raay TJ and Crease TJ: Partial mitochondrial DNA sequnce of the crustacean Daphnia pulex, Curr. Genet., Vol. 25, p.66-72, (1994)
doi:10.1007/BF00712970