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Full Length Article

DNA Barcoding of Genus *Empoasca* Walsh, 1862 (Hemiptera: Cicadellidae) Based on *COI* Gene Sequences

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Abstract

Small green leafhoppers (Genus *Empoasca*) comprise of several species, with similar morphology or largely similar morphological characteristics, thereby making the identification of the different species difficult. In this study, the 683-bp sequence of the mitochondrial cytochrome oxidase subunit I (*COI*) gene was used as a DNA barcode to accurately identify species of small green leafhoppers. Results showed that among the 683 bases of *COI* gene, 483 conserved sites, 192 variable sites, 182 parsimony-informative sites, and 10 singleton sites were present, accounting for 70.72%, 28.11%, 26.55% and 1.46% of the total bases, respectively. Of the *COI* sequence, AT content was 70.44%, and GC content was 29.56%, demonstrating a significant bias toward AT richness. The average genetic distance within species was 0.0116, and the average distance between species was 0.160, showing that the intraspecific differences are significantly lower than the interspecific differences and that obvious barcode gaps existed among the different species. Phylogenetic analysis showed that phylogenetic trees could distinguish well the five species of small green leafhoppers from each other; that is, five species of leafhoppers each formed an independent branch with a high degree of support (100% bootstrap value for two trees). The small green leafhoppers exhibited a significant "hot area" for intraspecific sequence alignment comparison, suggesting that the intraspecific similarity degree of the sequence was significantly higher than the interspecific similarity degree, making these results consistent with those of the phylogenetic analysis. The results indicated that short sequences of the *COI* gene could be used to identify the different species of small leafhoppers with similar morphology and provide a reliable method for the accurate identification of this genus. © 2019 Friends Science Publishers

Keywords: DNA barcoding; *Empoasca*; Identification; *COI* sequences; Phylogenetic analysis; Indicator vector analysis

Introduction

Small green leafhoppers belong to the Hemiptera, Cicadelloidea, Cicadellidae, and Typhlocybinae (Qin, 2003). This group is composed of many species and is widely distributed, with many species being important pests in agriculture, such as *Empoasca onukii* Matsuda (Wang, 2004; Mu *et al*., 2012; Bian *et al*., 2014; Zhang and Chen, 2015), *Empoasca decipiens* Paoli (Ebadah, 2002; Demirel and Yildirim, 2008; Parrella *et al*., 2008; Fathi *et al*., 2009; Galetto *et al*., 2011), and *Empoasca fabae* (Harris) (Murray *et al*., 2001; Medeiros and Tingey, 2006; DeLay *et al*., 2012), which cause severe economic losses to agriculture year-round. The morphological characteristics of some insects in this genus of small green leafhoppers are similar, with only minor differences. Therefore, identification of these insect species based on traditional morphological study is difficult; moreover, misidentification, involving synonyms or heteronyms, can occur (Ke *et al*., 2016). The accurate and rapid identification of species is not only greatly significant to the identification and classification of species but also is valuable to the timely control of pests, the formulation of comprehensive control strategies, and the discovery of new species.

DNA barcoding is a molecular biology technique for rapid species identification using DNA of standard target genes (Hebert and Gregory, 2005; Hein *et al*., 2018). Since the concept was proposed in 2003 (Hebert *et al*., 2003), the DNA barcoding technique has been widely used in studies on the environment, ecology, and food (Chase *et al*., 2005; Cadotte *et al*., 2008, 2009; Lin *et al*., 2009; Moura *et al*., 2010), especially in insect taxonomy, such as for the identification of insects of Lepidoptera (Hajibabaei *et al*., 2006), Diptera (Meier *et al*., 2006), and Hymenoptera (Schmidt *et al*., 2015). This technique has greatly promoted the rapid identification of insect species and the discovery of new species (Pauls *et al*., 2010; Zhou, 2014). The cytochrome oxidase subunit I (*COI*) gene fragment is

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widely used as a standard gene barcode for species identification (Luo *et al*., 2013). However, some studies have shown that, in some genus groups, 16S rDNA, 28S rDNA, and ITS are also suitable for species identification (Chesters *et al*., 2012; Dai *et al*., 2012; Zheng *et al*., 2014). Therefore, the study of different gene fragments is beneficial to the screening of better DNA barcodes for the identification of species and to the evaluation of the application of different gene fragments in insect taxonomy. Until now, few reports have existed on the identification of insect species and phylogeny studies for the Genus *Empoasca* (Qin, 2003; Fu *et al*., 2014; Liu *et al*., 2017).

In this study, molecular biology methods were used to investigate the rapid and accurate identification of insect species of the Genus *Empoasca* based on the gene 683-bp sequence of the mitochondrial *COI* and to construct MP and BI phylogenetic trees to study the phylogenetic relation among insects of the Genus *Empoasca*. This study evaluated the applicability of the *COI* gene as a DNA barcode gene fragment to distinguish and identify insect species of the Genus *Empoasca* and explores potential applications of a combined method of gene differentiation analysis, DNA barcoding gap analysis, phylogenetic analysis, and indicator vector analysis in species identification. DNA barcoding is expected to provide theoretical reference for the rapid and accurate identification of insect species of the Genus *Empoasca* and further study of phylogeny.

Materials and Methods

Collection of *E. onukii*

Empoasca onukii Matsuda was collected from Tea Convention Garden (107.48°E lon and 27.75°N lat), Meitan County, Zunyi City, Guizhou Province, in August 2015. Insect samples were collected using a random scavenging method. The collected insects were immediately placed in a test tube containing anhydrous ethanol. Seven days after collection, anhydrous ethanol was replaced in each tube, and then, the insects were stored in a -80°C freezer.

Extraction of Total DNA, Sequencing, and Alignment of the *COI* Gene Sequence from *E. onukii*

Five heads of *Empoasca onukii* were used for the experiments. A single *E. onukii* was placed in 1.5-mL centrifuge tube, an appropriate amount of liquid nitrogen was added, and the insect was ground completely with a sterilized rod. Total DNA was extracted using the Omega E.Z.N.A.™ Insect DNA extraction kit according to manufacturer's instructions. The total DNA was stored in a -80°C freezer.

For each sample, 1.5 μL of isolated total DNA was used for PCR amplification. The primers used for amplification were published by Takiya *et al*. (2006). The sequence of the forward primer was 5′-TTGATTTTTTGGTCAYCCWGAAGT-3′ and of the reverse primer was 5′-TTCATTGCACTAATCTGCCATACTA-3′. The total PCR reaction volume was 30 μL: 1 μL of each forward and reverse primer, 15 μL of 2X Taq PCR Master Mix, 1.5 μL of DNA template, and 11.5 μL of ddH_2O. PCR was performed as follows: 94°C initial denaturation for 3 min; 35 cycles of 94°C denaturation for 30 s, 50°C renaturation for 1 min, 72°C extension for 1 min; an additional extension for 10 min at 72°C; and heat preservation at 4°C. 5 μL of the PCR reaction product was analyzed by 1% agarose gel electrophoresis and was detected and photographed on a Gel-Doc imager to confirm fragment amplification. The remaining PCR product was sequenced by Bioengineering (Shanghai) Co., Ltd.

Sequencing results were aligned and compared using SeqMan 5.00 and MEGA 6.06. After the flanking sequence had been arranged at both ends, the arranged sequences were blasted for homology alignment on the NCBI website (National Center for Biotechnology Information) (Zhang *et al*., 2000).

Collection of Sequences from other Leafhopper Species of the Genus *Empoasca*

The sequences of other leafhopper species and outgroups were collected from the NCBI database. See Table 2 for detailed information.

Statistical Analysis

The taxonomic categories of Genus *Empoasca* were analyzed based on the genetic distance of the *COI* genes. DNA barcoding gap analysis used R software 3.4.0. Then, MEGA 6.06 was used to calculate the base composition of the mtDNA *COI* gene among leafhoppers of the Genus *Empoasca* (Tamura *et al*., 2013). Base substitution saturation analysis employed DAMBE 5.2.73 (Xia *et al*., 2003; Xia and Lemey, 2009). The MP, and BI phylogenetic trees were constructed using Paup 4b10, and MrBayes v3.2.6, respectively (Swofford, 2003; Ronquist *et al*., 2012). The barcode vector analysis of the *COI* gene was carried out using MATLAB R2016a with reference to the Sirovich *et al*. (2009) method.

Results

Sequence Analysis of Five Species of Leafhoppers from the Genus *Empoasca*

The registration numbers of *COI* sequences of *E. onukii* are detailed in Table 1. After PCR amplification, and NCBI database searching, the 25 *COI* sequences were obtained from the five species of leafhoppers from the Genus *Empoasca*. From these sequences, 683 homologous bases were used in the Mega 6.0 sequence comparison analysis, among which 483 conserved sites, 192 variable sites, 182

Table 1: Information about *E. onukii* samples

Species	Code	Genebank accession number	Length (bp)
E. onukii	CBY1	MH631465	701 bp
	CBY2	MH631466	689 bp
	CBY3	MH631467	689 bp
	CBY4	MH631468	691 bp
	CBY5	MH631469	691 bp

Table 2: Information about leafhoppers of the Genus *Empoasca* whose sequences were downloaded from NCBI database (except for *E. onukii*)

Species	Code	Gene accession number	Length (bp)
E. coccinea	BIOUG00941-H02	KR044841	658 bp
	BIOUG00906-E07	KR043778	658 bp
	BIOUG00999-F07	KR041873	658 bp
	BIOUG00999-F06	KR041300	658 bp
	BIOUG01012-B11	KR041254	658 bp
E. decipiens	BIOUG00806-A05	KR582209	658 bp
	BIOUG00891-C03	KR565381	658 bp
	BIOUG00891-E02	KR560273	658 bp
	BIOUG01487-B03	KR584202	658 bp
	BIOUG01487-D02	KR579805	658 bp
E. fabae	BIOUG03340-D08	KR044774	658 bp
	BIOUG01791-C06	KR043800	658 bp
	BIOUG01843-A03	KR033397	658 bp
	BIOUG01843-B02	KR036559	658 bp
	BIOUG02961-E11	KJ091046	658 bp
E. luda	BIOUG04209-D07	KR583335	658 bp
	BIOUG04646-D05	KR576003	658 bp
	BIOUG04209-D01	KR572692	658 bp
	HEM305488	KR043650	658 bp
	HEM305487	KR035641	658 bp
Amrasca biguttula	ABI	KJ867503	700 bp
Anaka burmensis	Hap1	KY320208	700 bp
Thampoa dansaiensis	TDA	JX020564	627 bp
Typhlocyba rosae	BIOUG00947-A01	KR583915	658 bp

parsimony-informative sites, and 10 Singleton sites were identified, accounting for 70.72%, 28.11%, 26.55% and 1.46% of the all bases, respectively. In addition, 25 insertion/deletion sites were identified, accounting for 3.66% of all bases. In the *COI* sequence, the proportions of base T, C, A and G were 44.41, 14.53, 26.03 and 15.03%, respectively. AT and GC contents accounted for 70.44 and 29.56%, respectively, showing a significant AT bias.

Based on K2P model, the intraspecific and interspecific genetic distances were calculated for the five species of *Empoasca*. Fig. 1 shows that the maximum value of intraspecific genetic distance was 0.0790. The minimum genetic distance between species was 0.1325. The difference in the intraspecific genetic distances was significantly lower than that of the interspecific genetic distances, and obvious barcode gaps were observed among the different species.

Base Substitution Saturation Analysis

Base substitution saturation test of *COI* sequences of Genus *Empoasca* showed that the Iss.c values of the symmetric tree and asymmetric tree were 0.7244 and 0.4244, respectively, and the Iss value was 0.1910 (Table 3–4), which was significantly lower than the Iss.c value (two-tailed test, $P > 0.01$), indicating that in this study, the sequence base substitution was not saturated. Therefore, 683 loci were suitable for the subsequent phylogenetic analysis.

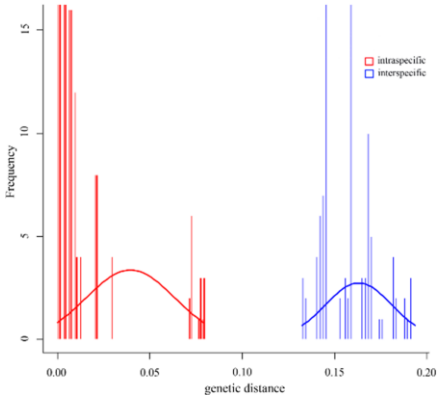

Fig. 1: DNA barcoding gap analysis. Intraspecific genetic distances: Minimum, 0; Median, 0.0046; Mean, 0.0116; Maximum, 0.0790; Interspecific genetic distances: Minimum, 0.1325; Median, 0.1589; Mean, 0.1600; Maximum, 0.1933

Phylogenetic Analysis

In this study, five species of leafhoppers from the Genus *Empoasca* and four outgroup species from four different genera were used to construct the MP and BI phylogenetic tree. As shown in Fig. 2, the topological structures of the two phylogenetic trees were basically similar, all two methods could effectively distinguish the *Empoasca* insects, and they were used to form five independent branches with a strong degree of support; bootstrap values of the MP phylogenetic tree were greater than 70% and posterior probabilities of the BI phylogenetic tree were all 100%. Moreover, the five species of the Genus *Empoasca* were easily distinguishable from the four outgroups: *Amrasca biguttula*, *Anaka burmensis*, *Thampoa dansaiensis*, and *Typhlocyba rosae*, and the support degree bootstrap value reached 72% and posterior possibilities value reached 100%. The results indicate that the *COI* gene barcode could accurately distinguish the *Empoasca* insects from the outgroup species.

Indicator Vector Analysis of *COI* Gene Fragments

The *COI* gene barcode vector map (Fig. 3) shows that the sequence alignments of the leafhoppers demonstrated significant intraspecific "hot regions", which indicate that the sequence similarity of within species was significantly higher than that between species. In addition, five species of *Empoasca* insects were effectively discriminated and there was a certain correlation between them. There were obvious differences in sequence similarity between the outgroup species and five species of *Empoasca* insects, and the correlation between them was low, therefore, the outgroup species was discriminated from five species of *Empoasca* insects, meanwhile, they were discriminated from each

Table 3: The results of false test of substitution saturation for a symmetrical tree

Prop. Invar. sites	Mean H	Standard Error	Hmax	Iss	Iss.c	T	DF	Prob (Two-tailed)	95% Lower Limit	95% Upper Limit
0.0000	0.3401	0.0194	1.7812	0.1910	0.7244	27.4965	607	0.0000	0.1529	0.2290

Table 4: The results of false test of substitution saturation for an extremely asymmetrical (and generally very unlikely) tree

Iss.c	T	DF	Prob (Two-tailed)	95% Lower Limit	95% Upper Limit
0.4244	12.0332	607	0.0000	0.1529	0.2290

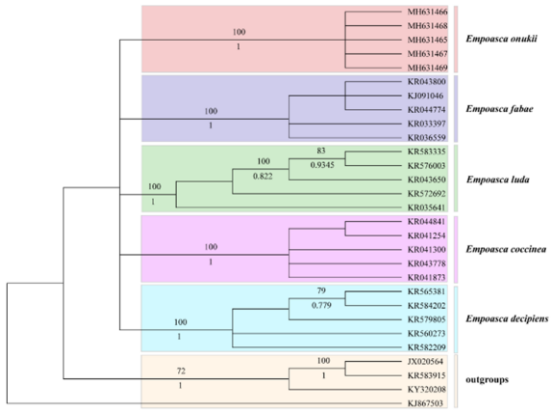

Fig. 2: The strict consensus phylogenetic tree was constructed with MP (Tree length=640, Consistency index (CI) =0.6141, Homoplasy index (HI) = 0.3859, Retention index (RI)=0.8270, Rescaled consistency index (RC)=0.5078) and BI (best model: HKY+I+G, -lnL=3840.2207) methods based on COI gene. Digits on the branch were MP bootstrap values (>70%), whereas Bayesian posterior possibilities (>90%) were under the branch

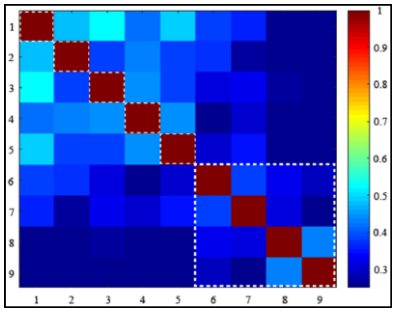

Fig. 3: Klee diagram for the COI gene. Color gradation in red indicates strong correlation (high correlation value). Red blocks in white dashed box indicate sequences of *E. fabae*, *E. luda*, *E. coccinea*, *E. decipiens*, *E. onukii* and outgroups
1. *E. fabae*; 2. *E. luda*; 3. *E. coccinea*; 4. *E. decipiens*; 5. *E. onukii*; 6. *Amrasca biguttula*; 7. *Anaka burmensis*; 8. *Thampoa dansaiensis*; 9. *Typhlocyba rosae*

other. These results clearly suggest that the intraspecific and interspecific genetic differences of the genus *Empoasca* are consistent with the results of the MP, and BI phylogenetic analysis.

Discussion

Among the 683 homologous bases of the *COI* gene from the five species of *Empoasca*, the AT content was much higher than the GC content, showing a significant AT bias, which is consistent with the characteristics of the mitochondrial DNA base composition of invertebrates (Zhang and Hewitt, 1997; Zhou *et al*., 2016). In the 25 sequences, 192 mutation sites were identified. The ratio of nucleotide conversion frequency to transversion was 3.642, and the conversion mainly occurred between the C and T bases, indicating that the phylogenetic relation of the five species of leafhoppers of the Genus *Empoasca* is close. This finding is consistent with the characteristics of nucleotide base substitution in the taxonomic order; that is, conversion is the main form of base substitution in closely related categories, and transversion is the main form in more remotely related categories (Simon *et al*., 1994; Kocher *et al*., 1989).

Ideally, the intraspecific DNA barcoding genetic distance of a species is lower than the interspecific distance, and a significant barcode gap should fall between intraspecific genetic distance and interspecific genetic distance (Köhler, 2007; Del-Prado *et al*., 2010), *i.e*., with a magnitude greater than 10 times (Hebert *et al*., 2003). In this study, no overlap of genetic distance was observed within the species and among the species for the five species of leafhopper of the Genus *Empoasca*. The average interspecific genetic distance was more than 13 times the intraspecific distance, and obvious barcode gaps were observed among the different species. The results suggest that the *COI* gene fragment could be used as the standard gene barcode to distinguish the insects of the Genus *Empoasca*. In addition, the results are consistent with the results reported by Deng *et al*. (2012), who used the *COI* gene DNA barcode to study six species of scales from the Genus *Ceroplastes* of the family Coccidae.

The aim of phylogenetic study is to deduce the phylogenetic relation and evolutionary relation among different genera (Trautwein *et al*., 2012). The results of phylogenetic analysis in this study showed that five leafhoppers of the Genus *Empoasca* formed independent branches with a high degree of support, indicating that the *COI* gene sequence fragments can accurately distinguish insects of the Genus *Empoasca*.

The MP, and BI trees could root the outgroups, indicating that the intraspecific insects of the Genus *Empoasca* were closely related to each other and distant from the outgroups, suggesting that these two phylogenetic trees could distinguish well the insects of the Genus

Empoasca from the outgroups and could be used for phylogenetic studies. The phylogenetic trees of MP and BI are slightly different in the branches of species of *Empoasca fabae*, the possible reasons for these discrepancies may be as follows: 1) the information contained in the single *COI* gene sequence fragment is limited (Zou and Song, 2008), 2) the rate of gene evolution among different groups is different (Degnan and Rosenberg, 2008), and 3) all methods used in phylogenetic analysis have certain limitations (Li *et al*., 2007). Moreover, in phylogenetic studies, only results with bootstrap value >70% or posterior probabilities >90% are reliable (Hillis, 1997; Miao *et al*., 2011). The specific reasons for this phenomenon need to be further studied using molecular biology methods.

The Klee diagram vector map can classify organisms with unknown sequences into groups of species and genera. However, it cannot predict the evolutionary state of the sequence (Stoeckle and Coffran, 2013). Lawrence *et al*. (2009) performed indicator vector analysis of 173 *COI* sequences from North American birds, and the results showed that 122 indicator vectors were correctly allocated to the different species (correct species assignment). The results of the indicator vector analysis of insects of the Genus *Empoasca* in this study show that the similarity of the intraspecific sequences was significantly higher than that of the interspecific sequences, and the genetic differences between the two groups were clearly visible. The correct species assignment was accomplished for the five species of leafhoppers in the Genus *Empoasca* and the four species of outgroups, indicating that the *COI* gene fragment was efficient in identification of insects of the Genus *Empoasca* and the outgroup species.

This study carried out differential analysis of the *COI* gene fragment and DNA barcoding gap analysis on insects of the Genus *Empoasca*, verified the results with phylogenetic analysis, and visualized the differences between intraspecific and interspecific genetic distance using Klee diagrams to create a vector map. These results demonstrate that the *COI* gene fragment could be used as a standard gene barcode for the identification of insects of the Genus *Empoasca* and indicate that the combination of the above techniques have great application potential in the field of insect species identification and phylogenetic research. Because the amount of information contained in a single gene fragment is limited and cannot fully satisfy the requirements of phylogenetic studies, the quantity and insect species of the Genus *Empoasca* should be increased in future studies, and more independent gene fragments should be used for combined analysis to obtain a more accurate evolutionary relation between insects of the Genus *Empoasca* and outgroups.

Conclusion

The results of this study provide an effective method for rapid and accurate identification of insect species of the Genus *Empoasca* based on a standard gene barcode.

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References

Bian, L., X.K. Sun, Z.X. Luo, Z.Q. Zhang and Z.M. Chen, 2014. Design and selection of trap color for capture of the tea leafhopper, *Empoasca vitis*, by orthogonal optimization. *Entomol. Exp. Appl.,* 151: 247–258

Cadotte, M.W., J. Cavender-Bares, D. Tilman and T.H. Oakley, 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS One*, 4: e5695

Cadotte, M.W., B.J. Cardinale and T.H. Oakley, 2008. Evolutionary history and the effect of biodiversity on plant productivity. *Proc. Natl. Acad. Sci.,* 105: 17012–17017

Chase, M.W., N. Salamin, M. Wilkinson, J.M. Dunwell, R.P. Kesanakurthi, N. Haider and V. Savolainen, 2005. Land plants and DNA barcodes: Short-term and long-term goals. *Phil. Trans. Royal Soc. B: Biol. Sci.,* 360: 1889–1895

Chesters, D., Y. Wang, F. Yu, M. Bai, T.X. Zhang, H.Y. Hu, C.D. Zhu, C.D. Li and Y.Z. Zhang, 2012. The integrative taxonomic approach reveals host specific species in an encyrtid parasitoid species complex. *PLoS One*, 7: e37655

Dai, Q.Y., Q. Gao, C.S. Wu, D. Chesters, C.D. Zhu and A.B. Zhang, 2012. Phylogenetic reconstruction and DNA barcoding for closely related pine moth species (*Dendrolimus*) in China with multiple gene markers. *PLoS One*, 7: e32544

Degnan, J.H. and N.A. Rosenberg, 2008. Discordance of Species Trees with Their Most Likely Gene Trees. *PloS Genet.,* 57: 131–140

DeLay, B., P. Mamidala, A. Wijeratne, S. Wijeratne, O. Mittapalli, J. Wang and W. Lamp, 2012. Transcriptome analysis of the salivary glands of potato leafhopper, *Empoasca fabae*. *J. Insect Physiol.,* 58: 1626–1634

Del-Prado, R., P. Cubas, H.T. Lumbsch, P.K. Divakar, O. Blanco, G.A.D. Paz, M.C. Molina and A. Crespo, 2010. Genetic distances within and among species in monophyletic lineages of Parmeliaceae (Ascomycota) as a tool for taxon delimitation. *Mol. Phylogenet. Evol.,* 56: 125–133

Demirel, N. and A.E. Yildirim, 2008. Attraction of various sticky color traps to *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and *Empoasca decipiens* Paoli (Homoptera: Cicadellidae) in cotton. *J. Entomol.,* 5: 389–394

Deng, J., F. Yu, T.X. Zhang, H.Y. Hu, C.D. Zhu, S.A. Wu and Y.Z. Zhang, 2012. DNA barcoding of six *Ceroplastes* species (Hemiptera: Coccoidea: Coccidae) from China. *Mol. Ecol. Resour.,* 12: 791–796

Ebadah, I.M.A., 2002. Population Fluctuations and diurnal activity of the leafhopper, *Empoasca decipiens* on some summer crops in Kalubia Governorate, Egypt. *Bull. Fac. Agric. Cairo Univ.,* 53: 653–670

Fathi, S.A.A., G. Nouri-Ganbalani and H. Rafiee-Dastjerdi, 2009. Life cycle parameters of *Empoasca decipiens* Paoli (Hom.: Cicadellidae) on four potato cultivars (*Solanum tuberosum* L.) in Iran. *J. Entomol.,* 6: 96–101

Fu, J.Y., B.Y. Han and Q. Xiao, 2014. Mitochondrial COI and 16sRNA evidence for a single species hypothesis of *E. vitis*, *J. formosana* and *E. onukii* in East Asia. *PLoS One*, 9: e115259

Galetto, L., C. Marzachì, S. Demichelis and D. Bosco, 2011. Host plant determines the phytoplasma transmission competence of *Empoasca decipiens* (Hemiptera: Cicadellidae). *J. Econ. Entomol.,* 104: 360–366

Hajibabaei, M., D.H. Janzen, J.M. Burns, W. Hallwachs and P.D.N. Hebert, 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proc. Natl. Acad. Sci.,* 103: 968–971

Hebert, P.D.N. and T.R. Gregory, 2005. The promise of DNA barcoding for taxonomy. *Syst. Biol.,* 54: 852–859

Hebert, P.D.N., S. Ratnasingham and J.R.D. Waard, 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Royal Soc. Lond. B: Biol. Sci.,* 270: 96–99

Hien, L.T.T., N.N. Linh, P.L.B. Hang, N.P. Mai, H.H. Hanh, H.T.T. Hue and H.V. Huan, 2018. Developing DNA barcodes for species identification of *Berberis* and *Dysosma* genera in Vietnam. *Intl. J. Agric. Biol*., 20: 1097–1106

Hillis, D.M., 1997. Phylogenetic analysis: Current biology. *Magazine,* 7: 129–131

Ke, Y.L., W.J. Wu, G.Y. Liu and Z.Q. Li, 2016. Genetic relationship between *Coptotermes ochraceus* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *J. Environ. Entomol.,* 38: 341–347

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F.X. Villablanca and A.C. Wilson, 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci.,* 86: 6196–6200

Köhler, F., 2007. From DNA taxonomy to barcoding–how a vague idea evolved into a biosystematic tool. *Zoosyst. Evol.,* 83: 44–51

Lawrence, S., M.Y. Stoeckle and Z. Yu, 2009. A scalable method for analysis and display of DNA sequences. *PLoS One*, 4: e7051

Li, Y.W., L. Yu and Y.P. Zhang, 2007. "Long-branch Attraction" artifact in phylogenetic reconstruction. *Yi Chuan*, 29: 659–667

Lin, S., H. Zhang, Y.B. Hou, Y.Y. Zhuang and L. Miranda, 2009. High-level diversity of dinoflagellates in the natural environment, revealed by assessment of mitochondrial cox1 and cob genes for dinoflagellate DNA barcoding. *Appl. Environ. Microbiol.,* 75: 1279–1290

Liu, J.H., C.Y. Sun, J. Long and J.J. Guo, 2017. Complete mitogenome of tea green leafhopper, *Empoasca onukii* (Hemiptera: Cicadellidae) from Anshun, Guizhou Province in China. *Mitochondr. DNA B*, 2: 808–809

Luo, Y.H., J. Liu, L.M. Gao and L.D. Zhu, 2013. Applications and advances of DNA barcoding in ecological studies. *Plant Divers. Resour.,* 35: 761–768

Medeiros, A.H. and W.M. Tingey, 2006. Glandular trichomes of *Solanum berthaultii* and its hybrids with *Solanum tuberosum* affect nymphal emergence, development, and survival of *Empoasca fabae* (Homoptera: Cicadellidae). *J. Econ. Entomol.,* 99: 1483–1489

Meier, R., K. Shiyang, G. Vaidya and P.K. Ng, 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Syst. Biol.,* 55: 715–728

Miao, H.E., L. Gao and K.E. Ling, 2011. Phylogenetic study of human parvovirus B19 distributed in China based on bayesian inference and maximum likelihood analysis. *Chin. J. Blood Transfus.,* 24: 399–401

Moura, C.J., D.J. Harris, M.R. Cunha and A.D. Rogers, 2010. DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zool. Scr.,* 37: 93–108

Mu, D., L. Cui, J. Ge, M.X. Wang, L.F. Liu, X.P. Yu, Q.H. Zhang and B.Y. Han, 2012. Behavioral responses for evaluating the attractiveness of specific tea shoot volatiles to the tea green leafhopper, *Empoaca vitis*. *Insect Sci.,* 19: 229–238

Murray, J.D., T.E. Michaels, K.P. Pauls and A.W. Schaafsma, 2001. Determination of traits associated with leafhopper (*Empoasca fabae* and *Empoasca kraemeri*) resistance and dissection of leafhopper damage symptoms in the common bean (*Phaseolus vulgaris*). *Ann. Appl. Biol.,* 139: 319–327

Parrella, G., S. Paltrinieri, S. Botti and A. Bertaccini, 2008. Molecular identification of phytoplasmas from virescent Ranunculus plants and from leafhoppers in Southern Italian crops. *J. Plant Pathol.,* 90: 537–543

Pauls, S.U., R.J. Blahnik, X. Zhou, C.T. Wardwell and R.W. Holzenthal, 2010. DNA barcode data confirm new species and reveal cryptic diversity in Chilean Smicridea (Smicridea) (Trichoptera: Hydropsychidae). *J. North Amer. Benthol. Soc.,* 29: 1058–1074

Qin, D.Z., 2003. *Taxonomic Study on Chinese Empoascici (Homoptera: Cicadellidae).* Northwest Sci-Tech University of Agriculture and Forestry, Shaanxi Sheng, China

Ronquist, F., M. Teslenko, P.V.D. Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard and J.P. Huelsenbeck, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.,* 61: 539–542

Schmidt, S., C. Schmid-Egger, J. Morinière, G. Haszprunar and P.D.N. Hebert, 2015. DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (H ymenoptera, Apoidea *partim*). *Mol. Ecol. Resour.,* 15: 985–1000

Simon, C., F. Frati, A. Beckenbach, P. Crespi, H. Liu and P. Flook, 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Amer.,* 87: 651–701

Sirovich, L., M.Y. Stoeckle and Y. Zhang, 2009. A scalable method for analysis and display of DNA sequences. *PLoS One*, 4: e7051

Stoeckle, M.Y. and C. Coffran, 2013. TreeParser-aided Klee diagrams display taxonomic clusters in DNA barcode and nuclear gene datasets. *Sci. Rep.,* 3: 1–6

Swofford, D.L., 2003. *PAUP*: Phylogenetic Analysis using Parsimony (*and other Methods) Version 4.0b 10*. Sinauer Associates, Sunderland, Massachusetts, USA

Takiya, D.M., P.L. Tran, C.H. Dietrich and N.A. Moran, 2006. Co-cladogenesis spanning three phyla: leafhoppers (Insecta: Hemiptera: Cicadellidae) and their dual bacterial symbionts. *Mol. Ecol.,* 15: 4175–4191

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.,* 30: 2725–2729

Trautwein, M.D., B.M. Wiegmann, R. Beutel, K.M. Kjer and D.K. Yeates, 2012. Advances in insect phylogeny at the dawn of the postgenomic era. *Annu. Rev. Entomol.,* 57: 449–468

Wang, N.W., 2004. Studies on the threshold and pesticide resistance comparison of green leafhopper *Empoasca vitis göthe. Master Thesis*. Fujian Agriculture and Forestry University, Fuzhou, China

Xia, X. and P. Lemey, 2009. Assessing substitution saturation with DAMBE//. *In*: *The Phylogenetic Handbook: a Practical Approach to DNA and Protein Phylogeny,* 2nd edition, Vol. 2, pp: 615–630. Philippe, L., M. Salemi and A.M. Vandamme (Eds.). Cambridge University Press, Cambridge, UK

Xia, X., Z. Xie, M. Salemi, L. Chen and Y. Wang, 2003. An index of substitution saturation and its application. *Mol. Phylogenet. Evol.,* 26: 1–7

Zhang, D.X. and G.M. Hewitt, 1997. Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. *Biochem. Syst. Ecol.,* 25: 99–120

Zhang, Z., S. Schwartz, L. Wagner and W. Miller, 2000. A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.,* 7: 203–214

Zheng, L.M., J.R. He, Y.S. Lin, W.Q. Cao and W.J. Zhang, 2014. 16S rRNA is a better choice than *COI* for DNA barcoding hydrozoans in the coastal waters of China. *Acta Oceanol. Sin.,* 33: 55–76

Zhou, N.N., M.X. Wang, L. Cui, X.X. Chen and B.Y. Han, 2016. Complete mitochondrial genome of *Empoasca vitis* (Hemiptera: Cicadellidae). *Mitochondr. DNA A*, 27: 1052–1053

Zhou, Q.S., 2014. *Application of DNA Barcoding Studies in Hymenopteran Parasitoids.* Anhui University, Hefei, China

Zou, X.H. and G. Song, 2008. Conflicting gene trees and phylogenomics. *J. Syst. Evol.,* 46: 795–807

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