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Original Article

Genetic structure of teak beehole borer, *Xyleutes ceramicus* (Lepidoptera: Cossidae), in northern Thailand

Atikan Panyamang,[a] Orawan Duangpakdee,[b] Atsalek Rattanawannee[a, *]

[a] *Department of Entomology, Faculty of Agriculture, Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak, Bangkok, 10900, Thailand*
[b] *Ratchaburi Campus, King Mongkut's University of Technology Thonburi, Bangmod, Thung Khru, Bangkok, 10140, Thailand*

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ABSTRACT

The teak beehole borer (*Xyleutes ceramicus* Walker) is one of the most serious insect pests of teak (*Tectona grandis* L.f.) in both natural forests and plantations in Thailand, especially in the northern part of the country. Larvae of *X. ceramicus* infest a living teak tree by burrowing a long tunnel in the trunk, which reduces the quality and value of the marketable timber. To determine the genetic structure of the species, the sequences were examined of two mitochondrial genes (*COI* and *16S rRNA*) and one nuclear gene (*EF1−alpha*) of 41 individuals collected from 14 teak plantation sites in northern Thailand. The results of the sequencing analysis indicated the presence of 32 mitochondrial and 8 nuclear haplotypes. The phylogenetic analysis results revealed that all haplotypes were highly interconnected and a weak phylogeographic structure was revealed. The low genetic distance (F_{ST}) and high per-generation female migration rate (*Nm*) of most population pairs suggested that long-distance dispersal and the absence of a geographic barrier to gene flow were significant factors that affected the demography of *X. ceramicus* in northern Thailand.

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Introduction

Teak (*Tectona grandis* L. f.) (Family Verbenaceae) is one of the most valuable sources of tropical timber in Asia because of the high quality of its wood (Gotoh et al., 2007; Tee, 1995). This species is endemic to south and southeast Asia, mainly Bangladesh, India, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka and Thailand (Gotoh et al., 2007). Because of the durability and decorative value of the wood, teak plantations have been established outside the natural distribution of the species in Africa, Latin America and the Caribbean (Ansari et al., 2012). In Thailand, teak plantations have increased to 1700 km^2 since the first plantation was established in 1906, especially in the northern part of the country where the plantation area has dramatically expanded (Gotoh et al., 2007). However, the teak plantations in Thailand have experienced decreased timber quality because of insect pest infestations, especially trunk and branch borers such as the teak canker grub (*Dihammus cervinus* Hope) and teak beehole borer (*Xyleutes ceramicus* Walker) (Gotoh et al., 2007; Hutacharern and Tubtim, 1995).

The teak beehole borer (*X. ceramicus*) belongs to the family Cossidae, Order Lepidoptera. It is considered as a major pest of teak in natural forests and plantations in Myanmar and Thailand (Hutacharern and Tubtim, 1995; Nair, 2007). Its distribution range extends over a vast zoogeographic area in southeast Asia, including Brunei, Indonesia, Malaysia, Myanmar, New Guinea, Philippines, Singapore and Thailand (Nair, 2007). *Xyleutes ceramicus* females deposit approximately 12,500 eggs, ranging from 3666 to 21,094 eggs (Gotoh et al., 2007), and the newly hatched larvae disperse on silk threads with the aid of the wind (Gotoh et al., 2007; Nair, 2007). The mature larva, having white and pink transverse bands on each segment (Fig. 1A), makes a tunnel in the wood that is approximately 2.5 cm in diameter and more than 20 cm in length (Gotoh et al., 2007). These tunnels in the timber accumulate over the lifetime of the tree and are not discovered until the tree is cut into logs (Fig. 1B). Although *X. ceramicus* larval attacks do not cause teak tree mortality, the attacks result in serious deterioration of the wood quality and market price in proportion to the number of holes in the tree (Gotoh et al., 2007; Nair, 2007).

Despite the fact that *X. ceramicus* is an economic problem because it causes serious degradation of teak timber, only a few

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* Corresponding author.
E-mail address: fagralr@ku.ac.th (A. Rattanawannee).

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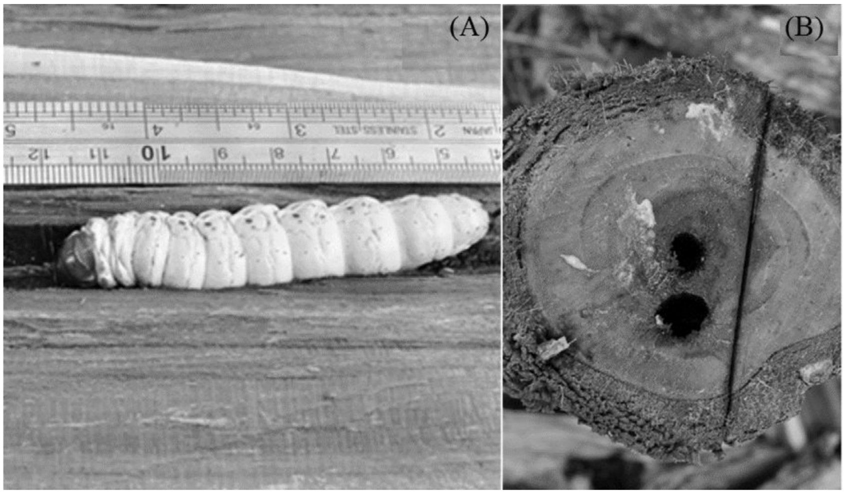

Fig. 1. (A) Larvae of *Xyleutes ceramicus* devastate living teak trees by burrowing a long tunnel; (B) holes made by *X. ceramicus* larvae in teak wood.

researchers have evaluated its genetic diversity within and between regional populations. Therefore, the objective of this study was to characterize the genetic structure of *X. ceramicus* populations collected from highly infested areas of teak plantations in northern Thailand using analyses based on sequences of mitochondrial and nuclear genes. This study should inform genetic variation among population of *X. ceramicus*, to develop more efficient management strategies in teak plantation in Thailand.

Materials and methods

Sample collection, DNA extraction, amplification and sequencing

Forty-one *X. ceramicus* larvae were collected from 14 teak plantation localities in northern Thailand (Table 1 and Fig. 2). All specimens were preserved in absolute ethanol, and were kept at −20 °C until required.

Total genomic DNA was extracted from the right hind true leg of individual larvae using a DNeasy® Blood & Tissue kit (Qiagen; Germantown, MD, USA) following the manufacturer's instructions. Two mitochondrial genes (mtDNA) were examined—cytochrome oxidase subunit I (*COI*) and 16S ribosomal RNA gene (*16S rRNA*)—and one nuclear gene (nDNA)—elongation factor-1 alpha (*EF1−alpha*)—for population genetic and phylogenetic analyses. Gene fragments were amplified using polymerase chain reaction (PCR) in a T100™ thermal cycler (BIO−RAD). Each PCR reaction was performed using a final volume of 40 μL, which contained 1 × PCR master mix (Fermentas Life Science; Vilnius, Lithuania), 20 μmol of each primer, and at least 1 μg of the genomic DNA template. The following PCR protocol was used: an initial denaturation step at 94 °C for 5 min; 35 cycles of 1 min at 94 °C, 1 min of annealing (case-by-case between 50 °C and 55 °C depending on the primer pair) and 2 min at 72 °C; and a final extension at 72 °C for 5 min. Primer names, references and primer sequences are shown in Table 2. The PCR products were purified using a QIAquick® Gel Extraction kit (Qiagen, Germantown, MD, USA), and were then directly sequenced by AITbiotech Pte. Ltd. (Singapore).

Ethics statement

The teak beehole borer (*Xyleutes ceramicus*) is one of the most serious insect pests of teak (*Tectona grandis* L.f.) in both natural forests and plantations. It is not an endangered species or protected by law. All experimental procedures using the animal conformed to the guidelines established by the Animal Experiment Committee, Kasetsart University, Thailand (Approval no. ACKU 60-AGR-002).

Sequence alignment and diversity indices

Sequences were initially aligned and edited for each gene separately using the MEGA6 v. 6.06 software (Tamura et al., 2013), and no gaps or indels were detected in the two mitochondrial genes. However, *EF1−alpha* contained a few indels, therefore automatic alignment in MEGA6 was conducted and followed by manual alignment and editing. All nucleotide sequences examined in this study were deposited in the GenBank database (Table 1). The following types of genetic variation were then investigated using DNAsp v. 5.0 (Librado and Rozas, 2009): average number of nucleotide differences (k), number of polymorphic (segregating) sites (S), number of haplotypes (No), haplotype diversity (h) (Nei, 1987) and nucleotide diversity (P_i) (Nei and Li, 1979).

Genetic distance and migration estimates

In this study, the genetic distance (F_{ST}) values were used to examine the genetic structure among *X. ceramicus* populations. The analysis of molecular variance (AMOVA) was used to determine the population genetic structure under grouping by province population (Excoffier et al., 1992). The significance of pairwise F_{ST} and AMOVA was calculated with 1000 permutations at a significance level of 0.05. These two procedures were performed using the ARLEQUIN v. 3.5.2.2 software (Excoffier and Lischer, 2010). In addition, the distances between DNA sequences were calculated using the Kimura-2-parameters method (Kimura, 1980). The per-generation female migration rate (N_m) was estimated using the pairwise migration rate based upon the following equilibrium relationship: $F_{ST} = 1/(2N_m + 1)$ (Li et al., 2006).

Historical demography

To examine the demographic history of *X. ceramicus*, Fu's *Fs* (Fu, 1997) and Tajima's *D* (Tajima, 1989) were calculated as assessments of possible population expansion by deviation from neutrality. These two statistics and the corresponding significance tests were calculated using the ARLEQUIN v. 3.5.2.2 software (Excoffier and Lischer, 2010) with 1000 bootstrap replicates.

Table 1
Xyleutes ceramicus collections from northern Thailand.

Location	*No*	Sample code	*N*	Approximate coordinates	GenBank accession number			Haplotype	
					COI	*16s rRNA*	*EF1−alpha*	*mt*	*n*
Mueang, Lampang	1	Lamp1	3	18° 30′ N 99° 37′ E	KU870526	KU870587	KX273175	Hap1	Hap1
	2	Lamp2			KU870527	KX343049	KU870552	Hap2	Hap1
	3	Lamp3			KU870528	KX343050	KU870553	Hap3	Hap1
Mae Moh, Lampan	4	Lamp5	3	18° 24′ N 99° 44′ E	KU870529	KU870588	KU870554	Hap4	Hap1
	5	Lamp6			KU870530	KU870589	KU870555	Hap5	Hap1
	6	Lamp7			KU870531	KU870590	KX273177	Hap6	Hap1
Thoen, Lampang	7	Lamp9	1	17° 31′ N 99° 20′ E	KU870532	KU870591	KX273178	Hap7	Hap1
Wang Chin, Phrae	8	Phrea15	3	17° 47′ N 99° 42′ E	KU870533	KX343051	KU870556	Hap8	Hap1
	9	Phrea18			KX928970	KU870592	KU870557	Hap4	Hap1
	10	Phrea21			KX928971	KU870593	KU870558	Hap4	Hap2
Song, Phrae	11	Phrea22	4	18° 33′ N 100° 09′ E	KU870537	KU870594	KU870559	Hap9	Hap3
	12	Phrea23			KX928972	KU870595	KU870560	Hap10	Hap1
	13	Phrea24			KU870538	KX343052	KU870561	Hap11	Hap1
	14	Phrea25			KX928973	KU870596	KU870562	Hap12	Hap1
Rong Kwang, Phrae	15	Phrea29	3	18° 21′ N 100° 49′ E	KU870539	KU870597	KX273179	Hap13	Hap3
	16	Phrea30			KU870540	KU870598	KU870563	Hap14	Hap1
	17	Phrea31			KX928974	KU870599	KU870564	Hap15	Hap1
Rong Kwang, Phrae	18	Phrea32	3	18° 17′ N 100° 25′ E	KU870541	KU870600	KU870565	Hap15	Hap1
	19	Phrea34			KU870542	KX343053	KU870566	Hap16	Hap4
	20	Phrea35			KU870543	KX343054	KU870567	Hap17	Hap1
Mae Sariang, Mae Hong Son	21	MHS37	1	18° 22′ N 98° 01′ E	KU870545	KU870602	KX273180	Hap19	Hap1
Li, Lamphun	22	Lamph36	4	17° 55′ N 98° 53′ E	KU870544	KU870601	KU870568	Hap18	Hap1
	23	Lamph40			KU870548	KX343055	KX273182	Hap20	Hap3
	24	Lamph41			KU870549	KX343056	KU870569	Hap21	Hap1
	25	Lamph43			KU870551	KX343057	KU870570	Hap20	Hap3
Mae Ai, Chiang Mai	26	ChiangM44	5	19° 24′ N 98° 50′ E	KX928976	KU870603	KU870571	Hap22	Hap5
	27	ChiangM45			KX928977	KU870604	KU870572	Hap23	Hap1
	28	ChiangM46			KX928978	KX343058	KU870573	Hap24	Hap1
	29	ChiangM47			KX928979	KX343059	KU870574	Hap25	Hap1
	30	ChiangM48			KX928980	KX343060	KU870575	Hap25	Hap1
Chai Prakan, Chiang Mai	31	ChiangM49	3	19° 43′ N 99° 15′ E	KX928981	KX343061	KU870576	Hap26	Hap6
	32	ChiangM50			KX928982	KX343062	KU870577	Hap27	Hap1
	33	ChiangM51			KX928983	KX343063	KU870578	Hap23	Hap1
Chiang Dao, Chiang Mai	34	ChiangM52	6	19° 22′ N 98° 56′ E	KX928984	KX343064	KU870579	Hap23	Hap1
	35	ChiangM53			KX928985	KX343065	KU870580	Hap28	Hap1
	36	ChiangM54			KX928986	KX343066	KU870581	Hap29	Hap7
	37	ChiangM55			KX928987	KX343067	KU870582	Hap29	Hap1
	38	ChiangM56			KX928988	KU870605	KU870583	Hap30	Hap1
	39	ChiangM57			KX928989	KU870606	KU870584	Hap31	Hap1
Mueang, Chiang Rai	40	ChiangR63	2	19° 59′ N 99° 43′ E	KX928990	KU870607	KU870585	Hap32	Hap8
	41	ChiangR64			KX928991	KU870608	KU870586	Hap21	Hap3

N is number of *X. ceramicus* samples collected from each location. Mitochondrial and nuclear (*EF1−alpha*) haplotypes are denoted as *mt* and *n*, respectively.

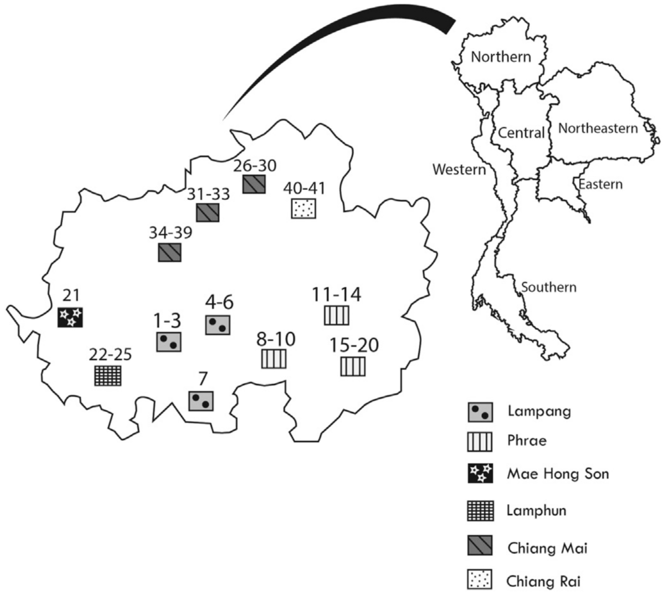

Fig. 2. Collection sites for *Xyleutes ceramicus* in northern Thailand, where numbers of the samples correspond to those in *No.* column of Table 1.

Additionally, the R_2 statistic (Ramos-Onsins and Rozas, 2002) was calculated using the DNAsp v. 5.0 software (Librado and Rozas, 2009) with 1000 coalescent simulations as the significance test. Analyses were performed for all samples and for each haplotype clade that was suggested by either phylogenetic or geographical analyses.

Phylogenetic and haplotype network analyses

Maximum likelihood (ML) and Bayesian inference (BI) methods were used to reconstruct the phylogenetic relationship among unique haplotypes. The Kakusan4 program (Tanabe, 2007) with maximum likelihoods calculated using Treefinder (Jobb et al., 2004) was used to evaluate the best-fit nucleotide substitution models as adjudicated by the Akaike information criterion (AIC) (Akaike, 1974) that was implemented in ML analyses and by the Bayesian information criterion (BIC) (Schwarz, 1978) that was implemented for BI analyses. The ML analysis was executed in Treefinder (Jobb et al., 2004) using the likelihood-ratchet method, and 1000 bootstrap replicates were used to estimate node confidence values. The BI analysis involved using the MRBAYES v. 3.1 software (Huelsenbeck and Ronquist, 2001), which employs a Metropolis-coupled Markov chain Monte Carlo (MC−MCMC) sampling approach, for phylogenetic reconstruction. The four-chain MC−MCMC analysis was performed twice in parallel for 3,000,000 generations, starting with a random tree, and trees were sampled every 100 generations. The initial 25% of the generations were discarded as "burn-in" samples. The remaining trees were used to examine the consensus tree topology, branch lengths and bipartition posterior probability (Huelsenbeck and Ronquist, 2001). Bipartition posterior probability values that were 0.95 or greater were considered significant (Larget and Simon, 1999). Three Lapidopteran species of the family Cossidae, *Zeuzara coffeae* (KJ508046), *Venessa indica* (mtDNA: GU372515 and *EF1−alpha*: GU372606), and *V. cardui* (mtDNA: GU372526 and *EF1−alpha*: GU372607), were used as outgroups.

A median-joining network (Bandelt et al., 1999) was also conducted for the nuclear *EF1−alpha* gene and a combined dataset of mitochondrial DNA using the program NETWORK (http://www.fluxustechnology.com). The median-joining network procedure uses a maximum parsimony approach to explore for the shortest phylogenetic trees of a given dataset (Bandelt et al., 1999).

Results

Sequence analysis

Under the optimal PCR conditions, *COI*, *16S rRNA*, and *EF1−alpha* fragment sizes were 658, 486, and 469 base pairs (bp) in length, respectively. The nucleotide composition of *COI* was A = 29.79%, T = 39.51%, G = 14.89% G and C = 15.81%. Similarly, high A + T content was detected in the mitochondrial *16S rRNA* sequence (A = 38.77%, T = 39.39%, G = 7.88% and C = 13.96%), but no A and T nucleotide bias was found in any of the nuclear *EF1−alpha* sequences (A = 25.37%, T = 24.52%, G = 21.54% and C = 28.57%). Therefore, A + T nucleotide bias in the animal mitochondrial genome (Boore, 1999) was also found in the *X. ceramicus COI* and *16s rRNA* gene sequences.

According to multi-alignment and pairwise sequence comparisons, 5 polymorphic sites of which 3 parsimony informative positions, 24 polymorphic sites of which 11 parsimony informative positions, and 7 polymorphic sites of which 2 parsimony informative positions were detected in *COI*, *16S rRNA* and *EF1−alpha* sequences, respectively (Table 3). The percentages of parsimony-informative characters relative to the total characters for each gene were: *COI* = 0.46%, *16s rRNA* = 2.63% and *EF1−alpha* = 0.43%. The frequency of transition/transversion mutations of *COI*, *16s rRNA*, and *EF1−alpha* were 80/20%; 33.33/66.66% and 57.14/42.86%, respectively. The polymorphic site analysis results supported the observation that mitochondrial genes exhibited higher mutation rates.

Population structure and haplotype divergence

A summary of the *X. ceramicus* molecular diversity indices is given in Table 4. Within the *X. ceramicus* samples, *COI*, *16s rRNA* and *EF1−alpha* had a total of 6, 27 and 8 unique haplotypes, respectively. The genetic diversity was estimated in terms of haplotype diversity (h) and nucleotide diversity (P_i); the estimate of haplotype diversity (h) of all samples inferred from nuclear *EF1−alpha* (0.457 ± 0.013) was lower than that of mitochondrial

Table 2
Details of primers used to amplify *COI*, *16S rRNA*, and *EF1−alpha* genes for *Xyleutes ceramicus* in this study.

Gene	Primer name	Sequence (5′−3′)	Annealing temperature (℃)	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	50	Folmer et al. (1994)
	Nancy	CCCGGTAAAATTAAAATATAAACTTC		Simon et al. (1994)
16S rRNA	LR-J-12887	CCGGTTTGAGCTCAGATCA	55	Zimmermann et al. (2000)
	LR-N-13398	CGCCTGTTTATCAAAAACAT		Zimmermann et al. (2000)
EF1−alpha	Ef51.9(f)-789	CARGACGTATACAAAATCGG	54	Monteiro and Pierce (2001)
	efrcM4(r)-1351	ACAGCVACKGTYTGYCTCATRTC		Monteiro and Pierce (2001)

Table 3
Parsimony information of polymorphic nucleotide sites among *COI*, *16s rRNA* and *EF1−alpha* haplotypes of *Xyleutes ceramicus* in Thailand. Only positions that are different from haplotype HAP1 are indicated.

	Nucleotide position	*COI*		*16s rRNA*										*EF1−alpha*	
		193	346	3	16	321	368	370	433	439	444	477	478	131	447
Haplotype	HAP1	T	A	T	A	G	G	G	T	G	G	G	A	T	G
	HAP2	.	.	.	.	.	.	.	.	.	.	.	T	.	C
	HAP3	C	.	.	.	A	A	.	.	.	A	.	T	.	.
	HAP4	C	G	.	.	A	A	A	.	A	A	.	T	.	.
	HAP5	C	G	.	.	.	.	.	.	.	.	.	T	C	.
	HAP6	C	.	.	.	A	A	.	G	.	.	.	T	.	.
	HAP7			.	.	.	.	.	G	.	.	.	T	.	T
	HAP8			.	.	.	A	.	.	.	.	.	T	C	.
	HAP9			A	T	A	A	.	.	.	A	.	T		
	HAP10			A	.	A	A	.	.	A	A	.	T		
	HAP11			A	T	.	.	.	.	.	A	.	T		
	HAP12			.	.	.	.	.	.	.	A	.	T		
	HAP13			.	.	.	.	.	.	.	.	.	.		
	HAP14			.	.	.	A	.	.	.	.	.	T		
	HAP15			.	.	.	.	.	.	.	A	.	T		
	HAP16			.	.	A	A	.	.	.	A	.	T		
	HAP17			.	.	A	A	.	.	A	A	A	T		
	HAP18			.	.	A	A	A	.	A	A	.	T		
	HAP19			.	.	A	A	A	.	A	A	.	.		
	HAP20			.	.	.	.	A	.	.	A	.	T		
	HAP21			.	.	A	.	.	.	A	A	.	T		
	HAP22			.	.	A	.	A	.	.	A	.	T		
	HAP23			.	.	A	.	.	.	A	A	A	T		
	HAP24			.	.	A	A	A	.	A	A	A	T		
	HAP25			.	.	A	.	.	.	.	A	.	T		
	HAP26			.	.	.	A	A	.	.	A	.	T		
	HAP27			.	.	.	A	A	.	A	A	.	T		

Table 4
Summary of molecular diversity indices and population expansion test statistics of two mitochondrial (*COI* and *16S rRNA*) genes and one nuclear (*EF1−alpha*) gene.

Gene	Locality	*N*	*No*	*S*	*k*	*h* (±SD)	P_i (±SD)	*D*	*Fs*	R_2
mtDNA	Lampang	7	7	14	5.524	1.000 (0.076)	0.0048 (0.0009)	−1.835	−2.773*	0.151
	Phrae	13	11	18	5.103	0.974 (0.039)	0.0045 (0.0007)	−0.510	−4.474*	0.146
	Lamphun	4	3	9	4.667	0.833 (0.222)	0.0041 (0.0016)	−0.492	1.355	0.326
	Chiang Mai	14	10	8	2.571	0.945 (0.045)	0.0022 (0.0003)	0.796	−5.324**	0.151
	Chiang Rai	2	2	1	1.000	1.000 (0.500)	0.0009 (0.0004)	/	/	/
	Mae Hong Son	1	1	/	/	/	/	/	/	/
	All samples	*41*	*32*	*29*	*4.995*	*0.987 (0.009)*	*0.0044 (0.0004)*	*−0.1836*	*−1.862**	*0.087*
EF1−alpha	Lampang	7	1	0	/	/	/	/	/	/
	Phrae	13	4	4	0.744	0.526 (0.153)	0.0016 (0.0006)	−1.437	−1.152	0.148
	Lamphun	4	2	1	0.667	0.667 (0.204)	0.0014 (0.0004)	1.633	−5.677**	0.333
	Chiang Mai	14	4	3	0.400	0.371 (0.153)	0.0009 (0.0004)	−1.685*	−2.369**	0.133
	Chiang Rai	2	2	3	3.000	1.000 (0.500)	0.0064 (0.0032)	/	/	/
	Mae Hong Son	1	/	/	/	/	/	/	/	/
	All samples	*41*	*8*	*7*	*0.606*	*0.457 (0.093)*	*0.0013 (0.0003)*	*−1.918**	*−5.677**	*0.064**

N = Number of individuals; *No* = number of haplotypes, *S* = number of polymorphic (segregation) sites, *k* = average number of nucleotide differences, *h* = haplotype diversity, P_i = nucleotide diversity. Fu's *Fs*, Tajima's *D*, and Ramos-Onsins' and Rozas' R_2 are indicated.
*$p < 0.05$; **$p < 0.01$.

COI (0.629 ± 0.046) and *16s rRNA* (0.971 ± 0.013). Moreover, the nucleotide diversity (P_i) of all samples was relatively low for all gene sequences, and it ranged from 0.0003 to 0.0105. The average number of nucleotide differences (k) in all samples was highest in *16s rRNA* (4.124), followed by *COI* (0.817) and *EF1−alpha* (0.606).

When the *COI* and *16S rRNA* were combined (1144 bp), 32 haplotypes were observed in 41 individuals. Among the 32 haplotypes, 25 were unique to a single individual, 5 were unique to individuals from the same population, and 2 were shared by individuals from different populations. The haplotype diversity (h), nucleotide diversity (P_i) and average number of nucleotide differences (k) of all samples were 0.987, 0.0044 and 4.995, respectively. Based on the combined mitochondrial genes, all populations exhibited high haplotype diversity (0.833–1.000) but low nucleotide diversity (0.0009–0.0048) (Table 4). Regarding mitochondrial genes, the ranges of sequence divergence among the 32 haplotypes, based on pairwise comparisons, were 0–0.6% (0–4 bp) in *COI* and 0.2–2.9% (1–13 bp) in *16S rRNA*. Similarly, the pairwise distance comparison among the eight nuclear haplotypes associated with nuclear *EF1−alpha* ranged from 0.2% (1 bp) to 0.9% (4 bp).

Historical demography

Assuming that *X. ceramicus* is a single population, the negative value of Fu's *Fs* was significant (−1.862), which indicated a recent population expansion. However, the relatively small positive value of Ramos-Onsins' and Rozas' R_2 (0.087) and the small negative value of Tajima's *D* were not significant (Table 4). A

Table 5
F_{ST} value and gene flow (*Nm*) among populations of *Xyleutes ceramicus* in northern Thailand. The data above and below the diagonal correspond to F_{ST} and *Nm*, respectively.

	Mitochondrial gene						*EF1−alpha*					
	Lampang	Phrae	Lamphun	Chiang Mai	Chiang Rai	Mae Hong Son	Lampang	Phrae	Lamphun	Chiang Mai	Chiang Rai	Mae Hong Son
Lampang	–	0.0342	0.1601*	0.3980**	0.2475	0.0334	–	−0.0274	0.7083	−0.0607	0.5865*	0.0000
Phrae	14.1241	–	0.0773	0.3224**	0.1223	0.0915	19.0185	–	0.1513	0.0168	0.2888	−0.9341
Lamphun	2.6230	5.9683	–	0.1548*	−0.0441	−0.0382	0.1386	2.8112	–	0.4616*	−0.0539	0.0000
Chiang Mai	0.7563	1.0509	2.7299	–	0.2737*	0.3574	8.6967	28.9118	0.5846	–	0.4815*	−1.0036
Chiang Rai	1.5202	3.5883	11.8378	1.3268	–	0.8188	0.3532	1.2301	9.9340	0.5395	–	−1.0057
Mae Hong Son	14.4701	4.9645	13.5890	0.8989	0.1106	–	inf	1.0353	inf	0.9985	0.9975	–

inf = infinite.
* $p < 0.05$; ** $p < 0.01$.

separate analysis suggested recent population expansion in Lampang, Phrae and Chiang Mai based on the significant negative Fu's *Fs* value, but the negative Tajima's *D* value and the positive Ramos-Onsins' and Rozas' R_2 value were not significant. None of the three values was significant with regard to Lamphun. Regarding *EF1−alpha*, the results indicated a population expansion based on the large negative Fu's *Fs* value (−5.677), the small positive Ramos-Onsins' and Rozas' R_2 value (0.063), and the relatively small negative Tajima's *D* value (−1.918), but a separate analysis revealed that these values were not significant, with the exception of Fu's *Fs* and Tajima's *D* values in Chiang Mai. In summary, most of the mitochondrial and nuclear genes of *X. ceramicus* in northern Thailand demonstrated signs of population expansion, but the examined values were not always significant. Only samples from Chiang Mai had a positive Tajima's *D* value, indicating a demographically stable population, but this value was not significant.

Genetic distance and migration estimates

Genetic distance (F_{ST}) and per-generation migration rates (*Nm*) between sites are shown in Table 5. Based on the mitochondrial genes, the pairwise F_{ST} among 15 pairs of *X. ceramicus* populations ranged from −0.0441 to 0.3980. The genetic differentiation of 10 pairs was not significant ($p > 0.05$), thus suggesting that several population pairs form a single genetic group. The samples from Chiang Mai showed significant differentiation for all pairs, with the exception of a pair from Mae Hong Son.

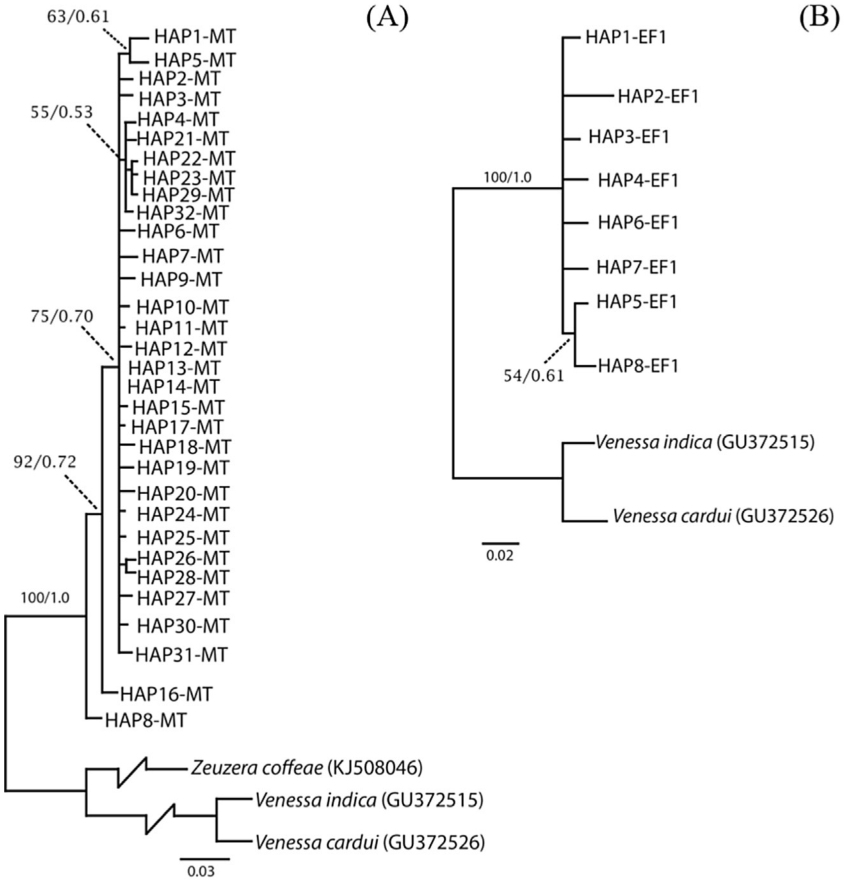

Fig. 3. Phylogenetic relationships of *Xyleutes ceramicus* in northern Thailand and outgroups based on Bayesian inference analysis of mitochondrial (*COI* and *16S rRNA*) (A) and nuclear (*EF1−alpha*) genes (B). For maximum likelihood (ML) and Bayesian inference (BI), nodal support inferred from Bayesian posterior probabilities and bootstrap values is shown as ML/BI.

Regarding *EF1−alpha*, three pairs, Chiang Mai-Lamphun (0.461), Chiang Mai-Chiang Rai (0.481) and Chiang Rai-Lampang (0.586), exhibited statistically significant genetic differentiation ($p < 0.05$). Regarding the per-generation female migration rate (*Nm*), the results indicated that more than one female *X. ceramicus* was estimated to migrate between population pairs per generation, and this result was applicable to most population pairs, including 12 pairs based on mitochondrial genes and 10 pairs based on nuclear genes.

Phylogenetic tree and haplotype network analysis

The best-fit models for ML tree reconstruction based on AIC were TIM_Gamma and J1ef_Gamma for mitochondrial and nuclear datasets, respectively, while the best-fit models for BI tree reconstruction based on BIC were GTR_Gamma and SYM_Gamma for mitochondrial and nuclear datasets, respectively. The phylogenetic trees estimated using the BI and ML methods exhibited congruent topologies for both mitochondrial and nuclear datasets. Because small differences were observed at the tips of some clades, only tree topologies from BI analyses are shown.

Phylogenetic relationships among haplotypes of *X. ceramicus* in Thailand are depicted in Fig. 3. The topology of the Bayesian tree inferred from mitochondrial and nuclear genes strongly supported the monophyly of *X. ceramicus* with an ML bootstrap value of 100% and a Bayesian posterior probability of 0.99−1.00. Most haplotypes were weakly associated or unresolved, and this might have been the result of small nucleotide differences among them. Based on mitochondrial gene sequences, haplotype groups HAP1−MT/HAP5−MT (63%) and HAP22−MT/HAP23−MT/HAP29−MT (55%) obtained marginal support as inclusive groups (Fig. 3A). However, only one haplotype group, HAP5−EF1/HAP8−EF1 (54%), was observed based on nuclear sequence data (Fig. 3B).

To further depict the genetic relationships among *X. ceramicus* haplotypes, median-joining networks were obtained, which search for all the shortest phylogenetic trees based on a maximum parsimony approach (Fig. 4). The mitochondrial network (Fig. 4A) showed that all unique haplotypes were highly interconnected with each other. The *EF1−alpha* network clearly displayed a star-like pattern with the most common haplotype (HAP1-EF1) in the star's center (Fig. 4B). Therefore, the networks suggested that no haplotype or haplotype group had diverged.

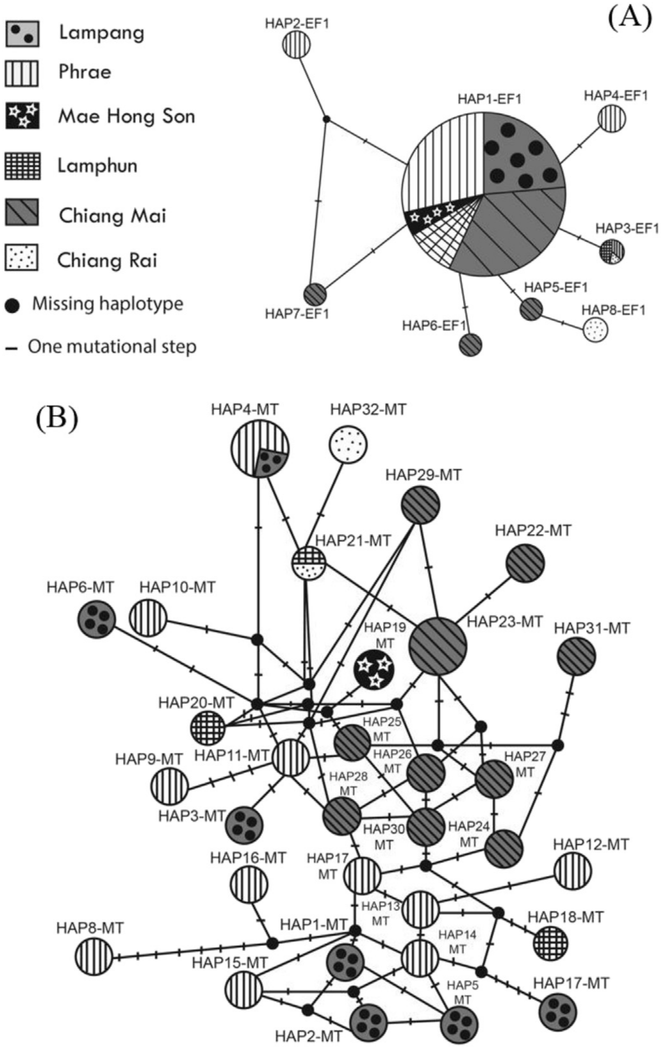

Fig. 4. Median-joining haplotype networks of *Xyleutes ceramicus* for mitochondrial (A) and nuclear (B) genes. Each circle represents one unique haplotype and the size of the haplotype circle corresponds to the abundance of individual samples. Each pattern demonstrates a sampling location. Dark spots and crossbars indicate missing haplotype and one mutational step, respectively.

Discussion

The genetic variability of *X. ceramicus* in Thailand was determined based on two mitochondrial genes (*COI* and *16S rRNA*) and one nuclear gene (*EF1−alpha*). The results indicated that the maximum DNA sequence divergence values, based on *COI*, *16S rRNA* and *EF1−alpha*, were 0.6%, 2.9% and 0.9%, respectively. This study represents the first examination of the genetic structure and genetic variation of *X. ceramicus*, which is the most important insect pest of teak plantation. Similar analyses of other Lepidopterans indicated that the maximum mitochondrial DNA sequence divergence was 0.2% in the domestic silkworm *Bombyx mori* (Kim et al., 2000), 0.9% in the diamondback moth *Plutella xylostella* (Li et al., 2006), 0.91% in the swallowtail butterfly *Papilio xuthus* and 1.67% in the cabbage butterfly *Pieris rapae* (Jeong et al., 2009), whereas the maximum sequence divergence was moderate to high in other insects. For instance, the estimates were 0.4% in the pea aphid *Acyrthosiphon pisum* (Boulding, 1998), 1.7% in the grain aphid *Sitobion avenae* (Xu et al., 2011), 1.2% in the mushroom fly *Coboldia fuscipes* (Bae et al., 2001), 3.8% in a phytophagous beetle of the genus *Ophraella* (Funk et al., 1995), 5.0% in the firefly *Pyrocoelia rufa* (Lee et al., 2003) and 11% in the oriental mole cricket *Gryllotalpa orientalis* (Kim et al., 2007). Therefore, the magnitude of sequence divergence in *X. ceramicus* is comparable to that in other Lepidopterans, while it is low to moderate compared to other insects.

Although the geographical coverage of the *X. ceramicus* sampling sites in the current study was not widespread, the mitochondrial and nuclear gene analyses detected diverse haplotypes. We found 32 mitochondrial haplotypes and 8 nuclear haplotypes in the 41 samples (Tables 1 and 4). However, the phylogenetic analysis revealed a weakly associated structuring clade among most haplotypes, and this was possibly the result of small nucleotide differences found in both the mitochondrial and nuclear datasets. In addition, there was no evidence of clustering based on the collection site in the tree. These results were supported by median-joining network analysis, which showed high interconnection among haplotypes (Fig. 4A and B). Further, the results of the population-based analysis also indicated that several *X. ceramicus* populations were genetically similar, and that moderately high gene flow rates existed between populations (Table 5). In addition, the mitochondrial DNA data indicated that most of the pairwise estimates of the per-generation female migration rate (*Nm*) were high (Table 5), which suggests there was population exchange between the sampling locations. Our results were in close agreement with previous studies of the diamondback moth (*P. xylostella*). Kim et al. (2000) reported that *P. xylostella* samples from Hawaii showed only one nucleotide difference as compared to Korean samples. Similarly, Li et al. (2006) found low genetic differentiation (F_{ST}) and a high rate of female migration (*Nm*) between Chinese *P. xylostella* populations. Generally, if $Nm < 1$, local populations will differentiate, but there will be little differentiation among populations if $Nm > 1$ (Wright, 1951). Based on our *Nm* values, the results suggested that dispersal over long distances and the absence of geographic barriers are major factors that affect the demography of the species.

The results of the nuclear *EF1−alpha* analyses indicated the occurrence of identical haplotypes over a wide range (Table 1). In particular, haplotype HAP1−EF1 was found at all surveyed localities (Table 1 and Fig. 4B), indicating low genetic diversity of *X. ceramicus* population in northern Thailand. Similar results were also reported for other insects, including the mushroom fly, *C. fuscipes* (Bae et al., 2001), grain aphid, *S. avenae* (Xu et al., 2011), diamondback moth, *P. xylostella* (Li et al., 2006; Niu et al., 2014), melon fly, *Bactrocera cucurbitae* (Hu et al., 2008), giant black aphid, *Pterochloroides persicae* (Kharrat et al., 2014) and cassava mealybug, *Phenacoccus manihoti* (Rattanawannee and Chongrattanameteekul, 2016). Analyses of the population genetic structure of the swallowtail butterfly, *Papilio xuthus*, and the cabbage butterfly, *Pieris rapae* showed that most populations are not genetically differentiated (Jeong et al., 2009). Phylogenetic analysis showed that all haplotypes of diamondback moth, *P. xylostella*, from China and Korea were highly interrelated and no discernible haplotype group was found (Jeong et al., 2009). Later, Niu et al. (2014) found similar results of low genetic diversity in *P. xylostella* collected from different localities in China, suggesting that population exchange and gene flow among the *P. xylostella* populations occurred. These results suggest that the occurrence of a common haplotype over such a wide area is noteworthy for many agricultural insect pest species.

The genetic diversity of a given species can be delimited with biogeographic history for a long-term perspective, and demographic processes provide a more immediate perspective (Avise, 1994). Several potentially important factors can impact demographic process in natural populations, and factors such as host plant, number of eggs laid, larval feeding and additional factors (Jeong et al., 2009) could play a major role in Lepidopteran species. Moreover, Avise et al. (1987) proposed a distribution pattern of haplotypes that could be summarized into a phylogenetic continuity that lacked geographic isolation and exhibited extensive distributions of close haplotypes. Therefore, a distribution was suggested for species that were widely connected within the species range or species that experienced recent historical interconnections through gene flow (Avise et al., 1987). In the present study, all examined populations were very closely related to each other, indicating the absence of geographic barriers that allow migration and gene flow to be maintained between populations.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

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