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Original Article

Mitochondrial DNA diversity of the lemon emigrant butterfly *Catopsilia pomona* Fabricius (Lepidoptera: Pieridae) in Khon Kaen Province, Thailand

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Abstract

We investigated genetic diversity, population structure, and demographic history of a *Catopsilia pomona* population in Khon Kaen Province, Thailand, using partial 863 base pairs *cytochrome oxidase subunit I* (*COI*) sequences among various sampling locations and periods. We collected 10 butterfly individuals from 3 locations and from 2 periods, before migration (March to April 2017) and after migration (May to June 2017). The sequence analyses of 60 butterflies revealed 36 haplotypes defined by 36 polymorphic sites. In the six groups we studied, the haplotype diversity in each group was high (0.667–0.978), whereas the nucleotide diversity was relatively low (0.002–0.006). The haplotype diversity and the number of haplotypes increased after migration in all locations. The Analysis of Molecular Variance (AMOVA) showed no differences between the groups before and after migration (Φ_{ct}=−0.030, P>0.9) and among sampling locations (Φ_{ct}=−0.001, P>0.4). The Bayesian skyline plot indicated a previous demographic expansion since the Middle Pleistocene.

Keywords: *cytochrome oxidase subunit I*, genetic diversity, migration, mitochondrial DNA, population structure

1. Introduction

Dispersal and gene flow are important processes for biological populations that not only increase genetic diversity but also counteract deleterious impacts of genetic drift and inbreeding (Frankham, Ballou, & Briscoe, 2010). It is expected that the gene flow among populations leads to an increase of a population's capacity to adapt to environmental changes by introducing new alleles into a population (Roitman *et al.*, 2017) and a reduction of genetic effects of population fragmentation such as inbreeding depression, loss of genetic variability or elevated extinction risk (Frankham *et al.*, 2010). Understanding the movement of organisms and their gene flow is thus crucial for the prediction of adaptive potential of a population to respond to environmental changes.

The lemon emigrant butterfly, *Catopsilia pomona*, is a medium-sized butterfly in the family of Pieridae. It is widely distributed throughout Asia, ranging from India, Southeast Asia, and China to northern Australia (Orr & Kitching, 2010; Inayoshi, 2017). *C. pomona* is one of the most abundant pierid butterflies in Southeast Asia (Corbet & Pendlebury, 1992). In Thailand, it can be found in all regions throughout the year (Subinprasert & Archawaranon, 2002). In Khon Kaen Pro-

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vince, this butterfly species can be observed almost year-round. It is very abundant in this area because many *Cassia fistula*, commonly known as golden rain trees, were planted in Khon Kaen Province as street trees and are important larval host plants of *C. pomona* (Ek-Amnuay, 2012; Chaianunporn & Khoosakunrat, 2018).

The common name of *C. pomona* reflects the migration behavior of this species. The migration has been observed in many areas, such as in India (Williams, 1927; Ramesh, Jahir, Satpathy & Selvanayagam, 2012), Australia (Dingle, Zalucki, & Rochester, 1999), and Thailand (Chaianunporn & Khoosakunrat, 2018). In Khon Kaen Province, the migration behavior of the butterfly was observed during June 2015 in eastward and westward directions (Chaianunporn & Khoosakunrat, 2018). However, the migration route of this butterfly in Thailand has never been studied. Research on the migration patterns and its influences on the genetic structure of the *C. pomona* populations are still limited and might hamper effective management planning for this species.

Molecular techniques make it possible to analyze demographic processes and migration among localities (Roderick, 1996; Freeland, 2005). Specifically, phylogeography and population genetics of a species can provide insight into how life history traits affect the patterns of genetic variation. By assessing landscape-level genetic diversity of a highly mobile butterfly species, scientists have gained a better understanding of how both natural and anthropogenic barriers have shaped their dispersal patterns (Stevens, Turlure & Baguette, 2010). As a wide-ranging, well dispersed butterfly species, it is expected that the population of *C. pomona* contains no or very low genetic structure among localities as observed in other highly mobile butterfly species, e.g., the monarch butterfly, *Danaus plexippus*, population which is panmictic in the entire North America (Pierce *et al.*, 2014; Zhan *et al.*, 2014; Pfeiler *et al.*, 2017).

Among molecular markers, mitochondrial DNA (mtDNA) sequences are used to study genetic diversity and phylogeography because of the relatively high mutation rate, haploid, maternal inheritance, lack of recombination, and their relatively conserved overall structure (Freeland, 2005). Moreover, it has been shown in *D. plexippus* that mtDNA sequences were suitable for studying genetic diversity in a highly mobile species (Brower & Jeansonne, 2004, Pfeiler *et al.*, 2017), and they provided consistent results with microsatellites (Lyons *et al.*, 2012; Pierce *et al.*, 2014), and whole genome analysis (Zhan *et al.*, 2014). In addition, the complete mitochondrial genome sequences of *C. pomona* have already been studied (Hao, Hao, Sun, Zhang, & Yang, 2014).

Here, we examined the genetic diversity, population structure, and demographic history of a *C. pomona* population using the partial DNA sequence of mtDNA gene *cytochrome oxidase subunit I* (*COI*). We collected *C. pomona* samples from three locations in Mueang Khon Kaen District, Khon Kaen Province, Thailand, and from two periods: before and after migratory events. The results provided information about the suitability of *COI* for studying the genetic variation within the population of *C. pomona* and the influence of the butterfly's migration on its genetic diversity and genetic structure.

2. Materials and Methods

2.1 Survey and sampling

We collected *C. pomona* from three locations in Mueang Khon Kaen District, Khon Kaen Province, Thailand where many *C. fistula* trees are planted and *C. pomona* have been observed: Ban Bueng Niam (BBN: east of Mueang Khon Kaen District: 16.438173°N 102.918382°E), Khon Kaen University (KKU: central of Mueang Khon Kaen District: 16.450637°N 102.816950°E), and Ban Nong Lub (BNL: west of Mueang Khon Kaen District: 16.450940°N 102.767773°E). BBN is about 10 kilometers away from KKU and KKU is about 5 kilometers away from BNL (Figure 1).

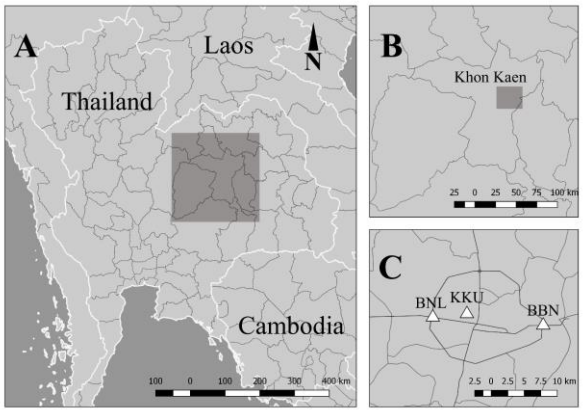

Figure 1. (A) Map of Thailand. The gray square indicates the area represented in Figure B. (B) Khon Kaen Province. The gray square indicates the area represented in Figure C. (C) Three study locations in Mueang Khon Kaen District, Khon Kaen Province, Ban Bueng Niam (BBN), Khon Kaen University (KKU) and Ban Nong Lub (BNL). BBN is about 10 kilometers away from KKU, and KKU is about 5 kilometers away from BNL. The solid lines present main roads of Mueang Khon Kaen District.

In June 2015, the migration behavior (directional flight in large groups) was detected via direct sighting study in BBN (Chaianunporn & Khoosakunrat, 2018). However, the migration time varies from year to year. In 2017, we simultaneously conducted weekly capture-mark-recapture in the three study locations and observed the migration behavior from late April to early May 2017. In addition, during this period, the number of *C. pomona* caught increased in all locations (Figure 2).

The sampling was conducted from March to June 2017 in all three sampling locations. The samples were divided into two sampling periods according to the migration behavior of the butterfly observed, i.e. before migration (BF) from March to April 2017 and after migration (AT) from May to June 2017. We collected 10 adult butterflies per location per period for a total of 60 samples. The butterfly samples were caught by net, immediately stored in 95% alcohol at −20 °C, and taken directly to the laboratory according to Moreau, Wray, Czekanski-Moir and Rubin (2013).

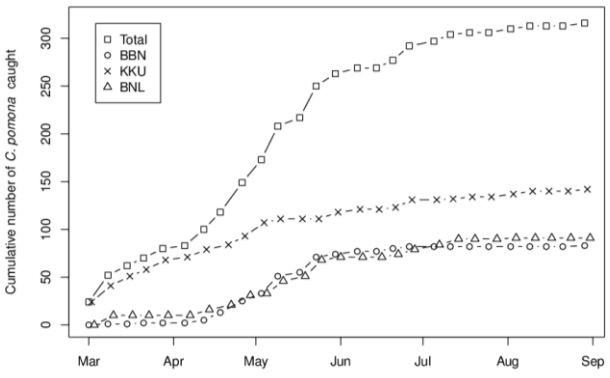

Figure 2. Cumulative number of C. pomona caught by week in a capture-mark-recapture study between March to September 2017 in BBN (circle line), KKU (cross line) and BNL (triangle line). The square line presents the weekly total cumulative number of three sampling locations.

2.2 Molecular analysis

Genomic DNA was extracted from the specimens' thoracic tissue using the ZR Tissue and Insect DNA Mini Prep™ (ZYMO RESEARCH). The DNA concentration and sample purity, determined from the 260 nm/280 nm ratio of each sample, was measured using the NanoDrop spectrophotometer. The partial *COI* gene was then amplified by the polymerase chain reaction (PCR). The forward primer and reverse primer were designed from the complete mtDNA sequence of *C. pomona* (NCBI Reference Sequence: NC_022687.1; Hao *et al.*, 2014) using Primer-BLAST (Ye *et al.*, 2012). The newly designed PCR forward primer called Cp COIF (5'-GGGGCAGGTACTGGATGAAC-3') and reverse primer called CpCOIR (5'-ATCGTCGAGGTA TTCCTGCT-3') are specific for the partial *COI* region of *C. pomona* mtDNA. This yielded a 958-bp sequence which corresponded to positions 1810–2767 of the reference sequence (NC_022687.1; Hao *et al.*, 2014). We prepared PCR Master Mix in a total volume of 30 µL containing 0.2 µL *Taq* DNA polymerase (5 u/µL), 3 µL 10x reaction buffer, 1.5 µL dNTPs (2 mM), 1.2 µL $MgCl_2$ (50 mM), 0.6 µL of each primer (10 µM), 2 µL of DNA templates, and 20.9 µL of distilled water. The final concentration of DNA templates ranged from 20 to 200 ng per reaction. The reaction conditions were performed as follows. The initial denaturation was at 95 °C for 5 min, followed by 40 cycles at 94 °C for 40 sec, annealing at 58.5 °C for 30 sec, and extension at 72 °C for 40 sec, with final extension at 72 °C for 5 min. The PCR products were checked by 1% agarose gel electrophoresis. We delivered the PCR products to Bioneer Corporation (Republic of Korea) for purification and sequencing using the same primer pair as we did for PCR. The sampling information of each specimen and its respective GenBank accession number is shown in Supplementary Table A1.

2.3 Data analysis

The sequences from the 60 samples were assembled and aligned using Bioedit Version 7.2.5 (Hall, 1999) and Clustal W multiple alignment. In order to analyze haplotype diversity, nucleotide diversity and shared haplotypes, the samples were divided according to 3 categories, i.e. 2 groups according to the sampling periods, 3 groups according to the sampling locations, and 6 groups according to both the sampling locations and the sampling periods. The genetic diversity and the analysis of molecular variance (AMOVA) were carried out using ARLEQUIN Version 3.5.2.2 (Excoffier & Lischer, 2010) to compare the values for Φ_{ct} (difference among groups), Φ_{sc} (difference among collections within groups), and Φ_{st} (differences among all collections) of the samples with all possible groupings. A neighbor joining tree based on pairwise Φ_{st} among *C. pomona* groups was built using ARLEQUIN. Analyses of the phylogenetic relationships among samples using maximum likelihood, neighbor joining, and maximum parsimony with 500 bootstrap replications were carried out by MEGA7 Version 7.0.26 (Kumar, Stecher, & Tamura, 2016). Bayesian interference analysis was performed with MrBayes v3.2 (Huelsenbeck & Ronquist, 2001). The program employs a Markov chain Monte Carlo (MCMC) sampling approach. A MCMC analysis was for 1 million generations starting with random tree and trees were sampled every 500 generations. Four hundred fifty generations were discarded as burn-in samples. The number of haplotypes and the haplotype diversity of each sampling location before and after migration were compared by Mann-Whitney U test using program R (version 3.4.3).

Median-joining networks (Bandelt, Forster, & Röhl, 1999) by haplogroups without pre- and post-processing steps were constructed by Network program (www.fluxus-engineering.com). The Bayesian skyline plots based on Bayesian MCMC analyses were created using BEAST 1.8.0. We ran jModel test 2.1.7 (Darriba, Taboada, Doallo, & Posada, 2012) and selected HKY+G as the best fitted substitution model according to the Akaike information criterion and Bayesian information criterion in order to choose the most suitable models for creating the BEAST input files by BEAUTi v1.8.2 (Drummond, Suchard, Xie, & Rambaut, 2012). The HKY+G model with strict clocks was used for the Bayesian skyline plot calculations. We assumed a neutral mutation rate of 1.15×10^{-9} per site per generation based on Pfeiler *et al.* (2017) by assuming 2.3% pairwise divergence per million years (Brower, 1994) and 10 generations of *C. pomona* per year (Chaianunporn & Khoosakunrat, 2018). The analysis was run for 8×10^{7} steps sampling every 10^{3} steps under the piecewise-linear Bayesian skyline model with a random starting tree. Tracer 1.6 was used to check for convergence.

3. Results and Discussion

3.1 Genetic diversity of *C. pomona*

In total, we analyzed sequences with a length of 863 bp of the partial *COI* gene of 60 *C. pomona* individuals and identified 36 unique haplotypes defined by 36 polymorphic sites (Table 1) (Supplementary Table A2). All nucleotide substitutions were synonymous transitions except at position 125 (haplotype 27) and 861 (haplotype 20) which resulted in neutral amino acid substitutions (Betts & Russell, 2003). Four haplotypes were found in more than one individual. Haplotype 1 was the most common haplotype among the butterfly samples (15 individuals, accounting for 25% of butterfly samples) and it matched with the reference sequence (NC_022687.1). It was found in all locations both before and

after migration (Figure 3). Haplotype 2 was found in 7 individuals in BF-BBN, AT–BBN, and AT-BNL, while haplotype 3 and 4 occurred in only one location, BF-KKU and AT-KKU, respectively. Thirty-two haplotypes occurred only in single individuals and distributed in various sampling locations and sampling periods. The phylogenetic relationship among all samples and supporting bootstrap and Bayesian interference values are presented in Supplementary Figure A1.

From a comparison between sampling periods, 15 haplotypes were found before migration and 23 haplotypes after migration (Table 1). The haplotype diversity (*H*) values were 0.869 and 0.968 for the before migration group and after migration group, respectively. The haplotype number and diversity were significantly higher in the after migration group than in the before migration group in all locations (Mann-Whitney U test: $U = 0.000$, P=0.043 for haplotype number and $U = 0.000$, P=0.046 for haplotype diversity). Among the three sampling locations, the highest number of haplotypes were found in KKU with 15 (7 before migration and 9 after migration), followed by 13 haplotypes in BNL (5 before migration and 9 after migration) and 11 haplotypes in BBN (5 before migration and 8 after migration). When we divided the groups into sampling locations and the periods (6 groups), the haplotype diversity was at its lowest in BF-BNL ($H = 0.667$) and its highest was in AT-KKU and AT-BNL ($H = 0.978$).

The haplotype diversity of *C. pomona* grouped according to the different sampling locations and periods corresponded to the migration behavior of *C. pomona* observed during the study period from late April to the beginning of May (Figure 2). An increase in the number of haplotypes and a higher haplotype diversity in *C. pomona* groups after migration suggested that migrating *C. pomona* individuals brought new haplotypes into the population.

The nucleotide diversity before and after migration was equal (π=0.004) (Table 1). Among the sampling locations, we found that the nucleotide diversity ranged from 0.003 (BBN) to 0.006 (KKU). Among the sampling locations and periods (6 groups), nucleotide diversity ranged from 0.002 (BF-BBN) to 0.006 (BF-KKU). The haplotype diversity and the nucleotide diversity in the combined dataset of all individuals of *C. pomona* was 0.925 and 0.004, respectively.

Table 1. Sequence analysis.

Group	n	k	PS	*H*±SD	π±SD
Before migration (BF)	30	15	24	0.869±0.050	0.004±0.003
After migration (AT)	30	23	25	0.968±0.022	0.004±0.003
BBN	20	11	14	0.868±0.057	0.003±0.002
KKU	20	15	22	0.958±0.033	0.006±0.003
BNL	20	13	17	0.853±0.080	0.004±0.002
BF-BBN	10	5	6	0.800±0.100	0.002±0.002
BF-KKU	10	7	15	0.867±0.107	0.006±0.004
BF-BNL	10	5	10	0.667±0.163	0.003±0.002
AT-BBN	10	8	10	0.956±0.059	0.004±0.002
AT-KKU	10	9	12	0.978±0.054	0.005±0.003
AT-BNL	10	9	13	0.978±0.054	0.004±0.003
All	60	36	36	0.925±0.026	0.004±0.003

Abbreviations: n, sample size; k, number of haplotypes; PS, number of polymorphic sites; *H*, haplotype diversity; SD, standard deviation; π, nucleotide diversity of each group of *C. pomona*; BBN, Ban Bueng Niam; KKU, Khon Kaen University; BNL, Ban Nong Lub.

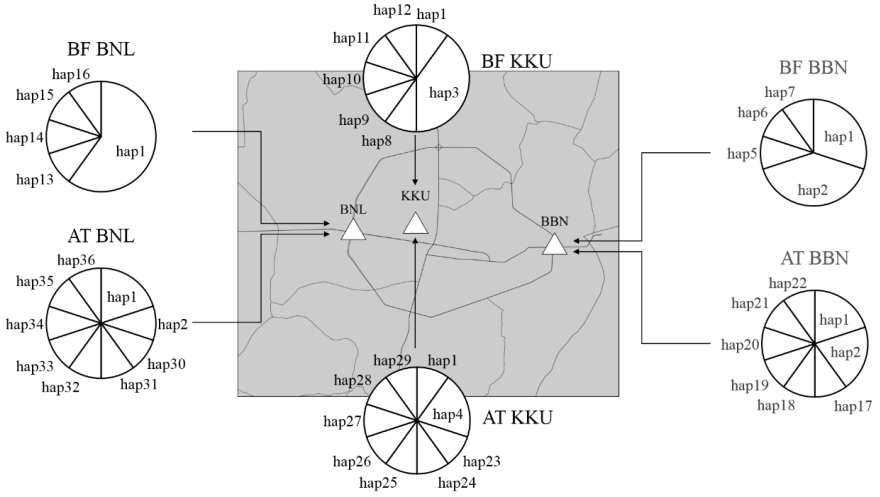

Figure 3. Pie diagrams showing the distribution of haplotypes of C. pomona in each sampling location and period. The numbers in the diagrams show the haplotype numbers.

The total haplotype diversity of *C. pomona* in this study (0.925) was relatively high in comparison with other butterfly populations, such as the satyrine butterfly, *Mycalesis orseis*, (0.550–0.890) (Benedick *et al.*, 2007), the afrotropical butterfly, *Bicyclus anynana* (0.750–0.920) (de Jong *et al.*, 2011), and the monarch butterfly, *Danaus plexippus* (0.220–0.600) (Pfeiler *et al.*, 2017). In contrast, the total nucleotide diversity was relatively low. This indicated there were only slight differences among the haplotypes. The combination of high haplotype diversity and low nucleotide diversity in the *C. pomona* population could be a sign of rapid demographic expansion from a small effective population size (Avise, 2000).

3.2 Neighbor joining tree among groups

The neighbor joining tree based on pairwise Φ_{st} values of groups shows that BF-BNL was closer to AT-BBN, and that BF-BBN was related to AT-BNL. The butterfly group in KKU was obviously located intermediately among BBN and BNL (Figure 4). This suggested migration of this species between BBN and BNL because haplotype 2 was shared by both BF-BBN and AT-BNL but it could not be found in BF-BNL. This result was consistent with the migration behavior observed by Chaianunporn and Khoosakunrat (2018) who reported the migration behavior of *C. pomona* at BBN in 2015 where *C. pomona* flew east-west and west-east directions.

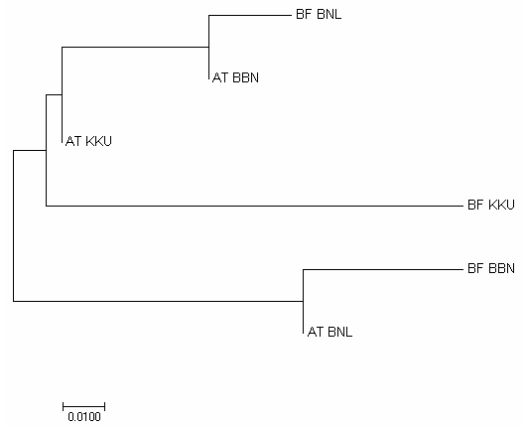

Figure 4. Neighbor joining tree based on pairwise Φ_{st} among 6 *C. pomona* groups defined by sampling locations and periods.

3.3 Analysis of molecular variance

The AMOVA results did not indicate any population structure between the before- and after-migration groups (−3.05% of variation, Φ_{ct}=−0.030, P>0.900), but there was significant genetic variation among collections within the same sampling periods (Φ_{sc}=0.110, P<0.003) and among all collections (Φ_{st}=0.083, P<0.002) (Table 2). Only 11.35% of the genetic variation was distributed among locations within the same sampling periods. The remaining 91.70% of the genetic variation was found among all collections. The AMOVA results based on varying sampling locations also revealed no significant difference among the sampling locations (−0.09% of variation, Φ_{ct}=−0.001, P>0.4), but significant genetic differentiation was found between the two sampling periods within the sampling locations (9.48% of variation, Φ_{sc}=0.095, P<0.013) and among all collections (90.61% of variation, Φ_{st}=0.094, P<0.000) (Table 3).

It was not unexpected that the AMOVA results suggested no genetic structure among the butterfly groups because *C. pomona* is a strong flyer and performs migration. A high migration rate could counteract the impact of genetic drift and therefore slow down genetic differentiation among populations living in fragmented habitats. This finding was in agreement with the results in other highly mobile butterfly species such as *D. plexippus*. Pfeiler *et al.* (2017) detected no genetic structure among migratory populations of *D. plexippus* in long-distance localities (over 100 km; Φ_{st}=−0.052–0.135 for *COI* and Φ_{st}=–0.064–0.072 for *COII*). In contrast, the population structure was observed among migratory and non-migratory populations of *D. plexippus* within the same distance (Φ_{st}=−0.265–0.613 for *COI* and Φ_{st}=0.423–0.469 for *COII*) (Pfeiler *et al.*, 2017).

Table 2. Analysis of Molecular Variance (AMOVA) of *C. pomona* grouping by sampling periods (periods to locations).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value	Fixation Indices
Among sampling periods	1	2.15	−0.06 Va	−3.05	>0.9	Φ_{ct}=−0.030
Among populations within sampling periods	4	15.53	0.22 Vb	11.35	<0.003	Φ_{sc}=0.110
Among all collections	54	93.70	1.74 Vc	91.70	<0.002	Φ_{st}=0.083
Total	59	111.38	1.89			

Table 3. Analysis of Molecular Variance (AMOVA) of *C. pomona* grouping by sampling locations (locations to periods).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value	Fixation Indices
Among sampling locations	2	7.03	−0.002 Va	−0.09	>0.4	Φ_{ct}=−0.001
Among populations within sampling locations	3	10.65	0.18 Vb	9.48	<0.013	Φ_{sc}=0.095
Among all collections	54	93.70	1.74 Vc	90.61	<0.000	Φ_{st}=0.094
Total	59	111.383	1.915			

3.4 Haplotype network and the Bayesian skyline plot

The haplotype network (Figure 5) showed that no population structure could be found between the groups before and after migration (Figure 5A) among the butterflies in different sampling locations (Figure 5B) or among both sampling periods and locations (Figure 5C) which reflected the results of AMOVA presented above. It showed the common haplotype (haplotype 1) found in all sampling locations and sampling periods with many single haplotypes around it. The star-like shape of the network indicated recent population expansion. The result was supported by the Bayesian skyline plot (Figure 6) which indicated a previous demographic expansion since the Middle Pleistocene (~750,000 years before present) followed by a stable effective female population size of 1.5×10^8 individuals over the last 200,000 years.

One reason for the large effective population size of *C. pomona* is that its larval host plants, *C. fistula* and other *Cassias* and *Sennas*, are common and very widespread. Its main host plant species, *C. fistula*, flourishes under tropical rainforest conditions and a tropical monsoon climate with low temperature tolerance to 0 ºC (CABI, 2018). The period when the butterfly population began to expand about 750,000 years to 1 million years ago (MYA) (Middle Pleistocene) corresponded to periodic climatic fluctuation (changes between interglacial and glacial cycles) as well as climate shifts of Indian summer monsoon around 1.1–1.8 MYA (Zhisheng *et al.*, 2011). These climatic events led to a warmer, more humid period. These climatic events were possibly related to increased habitable areas of the butterfly and expansion of *C. fistula* and other larval food plants from 750,000 years before present. As a consequence, *C. pomona* expanded its population during this time period. The similar patterns of population expansion during the Middle Pleistocene was also observed in another butterfly species in Asia, Fischer's blue butterfly, i.e. *Tongeia fischeri*, which experienced the same geological or climatic scenarios (Jeratthitikul *et al.*, 2013).

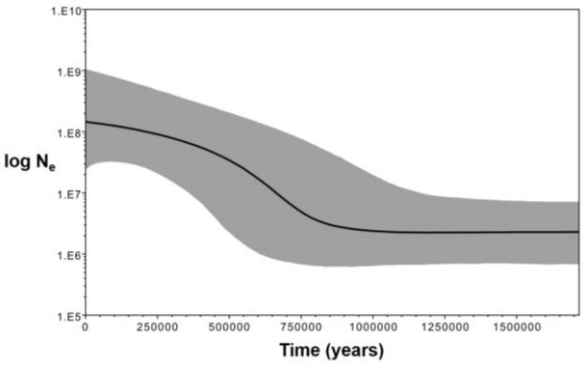

Figure 6. The Bayesian skyline plot inferred from the partial *COI* sequences shows the demographic history of *C. pomona* 1.7 million years ago. The middle solid line represents the median of effective female population size estimate of *C. pomona* (log N_e) over absolute time in years before present (X-axis) and gray shade indicates 95% highest posterior density intervals surrounding the median.

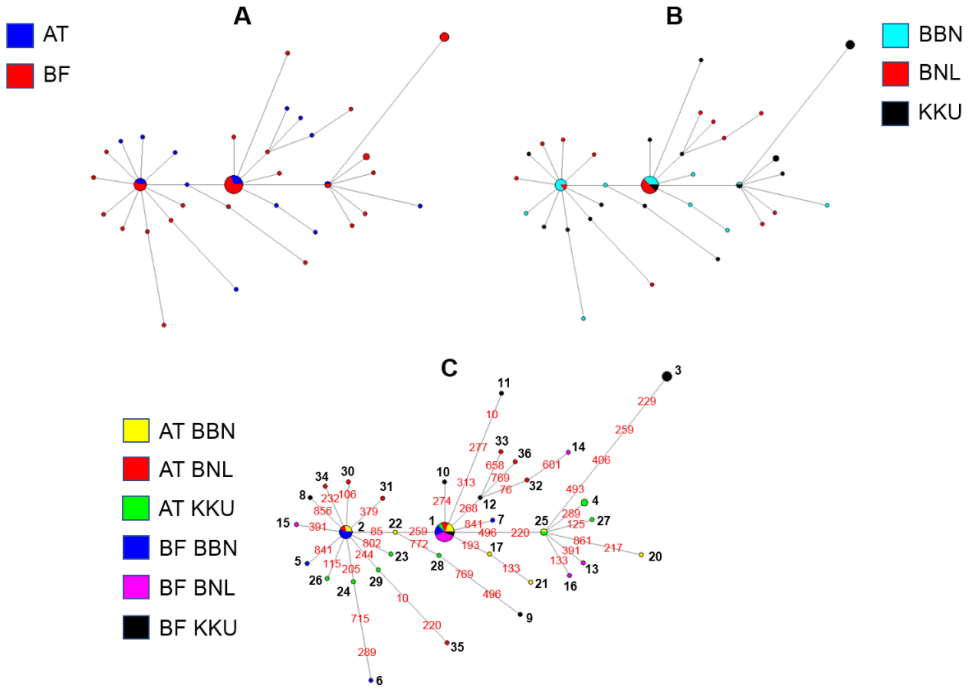

Figure 5. Network of 60 sequences of *C. pomona*. Circle size is relative to number of haplotype copies present in dataset. Colors indicate groups of the butterflies in this study: (A) Groups according to sampling periods; (B) Groups according to sampling locations; (C) Groups according to sampling periods and sampling locations. The black numbers indicate the haplotype number and the red numbers show the mutation point between each haplotype pair. The mutation points on the position 55, 259, and 841 were removed from the network because they caused reticulation of the network. Thus, haplotypes 18 and 19 are merged with haplotypes 25 and 1, respectively.

4. Conclusions

The molecular approach has been widely used to assess genetic diversity and population connectivity in species capable of dispersing over a wide geographic range, e.g., de Jong *et al.* (2011) and Pfeiler *et al.* (2017). Our results verify that the highly variable *COI* sequences are suitable to assess the genetic consequences of migration behavior of *C. pomona*. With this molecular marker, we observed that the migrating butterflies brought new alleles into the population. However, as a result of long-distance dispersal, there was no genetic structure of *C. pomona* detected at the scale of this study (about 15 kilometers). Thus, a further study of the butterfly in a wider geographical range based on this genetic marker would provide more insight into the population connectivity which would suggest the migration route and migration distance of this butterfly species. Moreover, additional molecular markers with different modes of inheritances should be examined to compare results between maternal, paternal, and bi-parental relationships. A systematic genetic monitoring based on different markers could provide a better understanding of the impacts of both the climatic fluctuations, and natural and human-made barriers on population structure. These factors are important for making an inference about the population status and providing information for management decisions.

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Appendix

Table A1. Sampling information of each *C. pomona* specimen, and its respective GenBank accession number. Sexes and forms are identified according to Ek-Amnuay (2012).

Code	Specimen No.	Locality	Coordinates	Date	Sex and form	Haplotype	GenBank accession No.
BF BBN 1	NK031	Ban Bueng Niam	16.438537°N, 102.918382°E	18-03-17	female f. *nivescens*	2	MH557302
BF BBN 2	NK039	Ban Bueng Niam	16.438242°N, 102.918323°E	08-04-17	female f. *jugurtha*	1	MH557303
BF BBN 3	NK043	Ban Bueng Niam	16.438255°N, 102.918377°E	08-04-17	male f. *alcmeone*	1	MH557304
BF BBN 4	NK042	Ban Bueng Niam	16.438249°N, 102.918383°E	08-04-17	female f. *nivescens*	2	MH557305
BF BBN 5	NK044	Ban Bueng Niam	16.438234°N, 102.918301°E	08-04-17	male f. *alcmeone*	2	MH557306
BF BBN 6	NK028	Ban Bueng Niam	16.438300°N, 102.918423°E	11-03-17	male f. *alcmeone*	5	MH557307
BF BBN 7	NK029	Ban Bueng Niam	16.438229°N, 102.918407°E	11-03-17	female f. *nivescens*	2	MH557308
BF BBN 8	NK040	Ban Bueng Niam	16.438261°N, 102.918396°E	08-04-17	female f. *crocale*	6	MH557309
BF BBN 9	NK041	Ban Bueng Niam	16.438249°N, 102.918383°E	08-04-17	male f. *alcmeone*	1	MH557310
BF BBN 10	NK045	Ban Bueng Niam	16.438251°N, 102.918380°E	08-04-17	male f. *alcmeone*	7	MH557311
BF KKU 1	NK012	Khon Kaen University	16.450669°N, 102.817024°E	01-03-17	male f. *alcmeone*	3	MH557312
BF KKU 2	NK017	Khon Kaen University	16.450622°N, 102.817152°E	01-03-17	male f. *alcmeone*	8	MH557313
BF KKU 3	NK018	Khon Kaen University	16.450493°N, 102.817152°E	01-03-17	male f. *alcmeone*	3	MH557314
BF KKU 4	NK020	Khon Kaen University	16.450512°N, 102.817158°E	01-03-17	male f. *alcmeone*	3	MH557315
BF KKU 5	NK013	Khon Kaen University	16.450645°N, 102.817045°E	01-03-17	male f. *alcmeone*	9	MH557316
BF KKU 6	NK014	Khon Kaen University	16.450684°N, 102.816988°E	01-03-17	male f. *alcmeone*	3	MH557317
BF KKU 7	NK015	Khon Kaen University	16.450670°N, 102.817015°E	01-03-17	male f. *alcmeone*	10	MH557318
BF KKU 8	NK016	Khon Kaen University	16.450728°N, 102.817045°E	01-03-17	female f. *nivescens*	1	MH557319
BF KKU 9	NK019	Khon Kaen University	16.450493°N, 102.817152°E	01-03-17	female f. *nivescens*	11	MH557320
BF KKU 10	NK021	Khon Kaen University	16.450512°N, 102.817158°E	01-03-17	female f. *nivescens*	12	MH557321
BF BNL 1	NK034	Ban Nong Lub	16.451310°N, 102.767508°E	02-04-17	male f. *alcmeone*	13	MH557322
BF BNL 2	NK036	Ban Nong Lub	16.451360°N, 102.767509°E	02-04-17	male f. *alcmeone*	14	MH557323
BF BNL 3	NK032	Ban Nong Lub	16.451246°N, 102.767509°E	02-04-17	male f. *alcmeone*	1	MH557324
BF BNL 4	NK033	Ban Nong Lub	16.451367°N, 102.767458°E	02-04-17	female f. *jugurtha*	15	MH557325
BF BNL 5	NK035	Ban Nong Lub	16.451315°N, 102.767508°E	02-04-17	female f. *jugurtha*	1	MH557326
BF BNL 6	NK037	Ban Nong Lub	16.451350°N, 102.767505°E	02-04-17	female f. *jugurtha*	1	MH557327
BF BNL 7	NK038	Ban Nong Lub	16.451368°N, 102.767460°E	02-04-17	female f. *jugurtha*	1	MH557328
BF BNL 8	NK046	Ban Nong Lub	16.450510°N, 102.767643°E	10-04-17	female f. *crocale*	16	MH557329
BF BNL 9	NK047	Ban Nong Lub	16.450500°N, 102.767651°E	10-04-17	male f. *alcmeone*	1	MH557330
BF BNL 10	NK048	Ban Nong Lub	16.450453°N, 102.767465°E	17-04-17	female f. *jugurtha*	1	MH557331
AT BBN 1	NK049	Ban Bueng Niam	16.438387°N, 102.918390°E	20-05-17	male f. *alcmeone*	17	MH557332
AT BBN 2	NK050	Ban Bueng Niam	16.438370°N, 102.918350°E	20-05-17	male f. *alcmeone*	18	MH557333
AT BBN 3	NK051	Ban Bueng Niam	16.438380°N, 102.918349°E	20-05-17	male f. *alcmeone*	2	MH557334
AT BBN 4	NK052	Ban Bueng Niam	16.438408°N, 102.918371°E	27-05-17	male f. *alcmeone*	1	MH557335

Table A1. Continued.

Code	Specimen No.	Locality	Coordinates	Date	Sex and form	Haplotype	GenBank accession No.
AT BBN 6	NK054	Ban Bueng Niam	16.438421°N, 102.918375°E	03-06-17	male f. *alcmeone*	20	MH557337
AT BBN 7	NK055	Ban Bueng Niam	16.438418°N, 102.918361°E	03-06-17	male f. *alcmeone*	2	MH557338
AT BBN 8	NK056	Ban Bueng Niam	16.438389°N, 102.918393°E	17-06-17	male f. *alcmeone*	21	MH557339
AT BBN 9	NK057	Ban Bueng Niam	16.438390°N, 102.918385°E	17-06-17	male f. *alcmeone*	22	MH557340
AT BBN 10	NK058	Ban Bueng Niam	16.438387°N, 102.918388°E	17-06-17	male f. *alcmeone*	1	MH557341
AT KKU 1	NK059	Khon Kaen University	16.450641°N, 102.816701°E	21-05-17	male f. *alcmeone*	4	MH557342
AT KKU 2	NK060	Khon Kaen University	16.450691°N, 102.816651°E	21-05-17	male f. *alcmeone*	23	MH557343
AT KKU 3	NK061	Khon Kaen University	16.450650°N, 102.816700°E	21-05-17	male f. *alcmeone*	24	MH557344
AT KKU 4	NK062	Khon Kaen University	16.450640°N, 102.816698°E	28-05-17	female f. *jugurtha*	25	MH557345
AT KKU 5	NK063	Khon Kaen University	16.450639°N, 102.816655°E	28-05-17	male f. *alcmeone*	1	MH557346
AT KKU 6	NK064	Khon Kaen University	16.450661°N, 102.816945°E	11-06-17	male f. *alcmeone*	4	MH557347
AT KKU 7	NK065	Khon Kaen University	16.450670°N, 102.816950°E	11-06-17	male f. *alcmeone*	26	MH557348
AT KKU 8	NK066	Khon Kaen University	16.450680°N, 102.816949°E	25-06-17	male f. *alcmeone*	27	MH557349
AT KKU 9	NK067	Khon Kaen University	16.450669°N, 102.816951°E	25-06-17	male f. *alcmeone*	28	MH557350
AT KKU 10	NK068	Khon Kaen University	16.450639°N, 102.816930°E	25-06-17	male f. *alcmeone*	29	MH557351
AT BNL 1	NK069	Ban Nong Lub	16.451303°N, 102.767456°E	22-05-17	male f. *alcmeone*	30	MH557352
AT BNL 2	NK070	Ban Nong Lub	16.451314°N, 102.767439°E	22-05-17	male f. *alcmeone*	2	MH557353
AT BNL 3	NK071	Ban Nong Lub	16.451300°N, 102.767421°E	22-05-17	male f. *alcmeone*	1	MH557354
AT BNL 4	NK072	Ban Nong Lub	16.451325°N, 102.767431°E	22-05-17	male f. *alcmeone*	31	MH557355
AT BNL 5	NK073	Ban Nong Lub	16.451333°N, 102.767419°E	22-05-17	male f. *alcmeone*	32	MH557356
AT BNL 6	NK074	Ban Nong Lub	16.451329°N, 102.767440°E	05-06-17	male f. *alcmeone*	33	MH557357
AT BNL 7	NK075	Ban Nong Lub	16.451300°N, 102.767455°E	05-06-17	male f. *alcmeone*	34	MH557358
AT BNL 8	NK076	Ban Nong Lub	16.451310°N, 102.767443°E	05-06-17	male f. *alcmeone*	35	MH557359
AT BNL 9	NK077	Ban Nong Lub	16.451313°N, 102.767445°E	19-06-17	male f. *alcmeone*	36	MH557360
AT BNL 10	NK078	Ban Nong Lub	16.451322°N, 102.767430°E	19-06-17	male f. *alcmeone*	1	MH557361

Table A2. Nucleotide variability among the 36 haplotypes of the partial COI gene obtained from 60 individuals of C. pomona. The sequence of NC_022687.1 (Hao *et al.*, 2014) was used as reference sequence and matches haplotype 1. The number of individuals with each haplotype is given in parentheses. Dots indicate where match nucleotides with the reference sequence occur in sequences. Nucleotide position numbers indicate the positions of polymorphic sites along the 863 base pair sequence.

	Nucleotide position																	
Haplotype	10	55	76	85	106	115	125	133	193	205	217	220	229	232	244	259	268	274
1(15)	C	G	A	C	T	T	G	C	A	T	T	G	A	C	T	C	G	A
2(7)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.
3(4)	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.	T	.	.
4(2)	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
5(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.
6(1)	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	T	.	.
7(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.
9(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
10(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
11(1)	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table A2. Continued.

	Nucleotide position																	
Haplotype	10	55	76	85	106	115	125	133	193	205	217	220	229	232	244	259	268	274
12(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
13(1)	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
14(1)	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
15(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.
16(1)	.	A	.	.	.	.	.	T	.	.	.	A	.	.	.	.	.	.
17(1)	.	A	.	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.
18(1)	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
19(1)	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
20(1)	.	A	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.
21(1)	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
22(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
23(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.
24(1)	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	T	.	.
25(1)	.	A	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
26(1)	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.	T	.	.
27(1)	.	A	.	.	.	.	A	.	.	.	.	A	.	.	.	.	.	.
28(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
29(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	T	.	.
30(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.
31(1)	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	T	.	.
32(1)	.	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
33(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
34(1)	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	T	.	.
35(1)	T	.	.	T	.	.	.	.	.	.	.	A	.	.	C	T	.	.
36(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.

Haplotype	277	289	313	379	391	406	493	496	601	658	715	769	772	802	820	841	856	861
1(15)	C	T	T	A	G	T	T	C	T	C	A	G	A	G	C	C	A	A
2(7)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3(4)	.	.	.	.	.	C	C	T	.	.	.	.	.	.	.	.	.	.
4(2)	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
5(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
6(1)	.	C	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
7(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
8(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.
9(1)	.	.	.	.	.	.	.	T	.	.	.	A	G	.	.	.	.	.
10(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11(1)	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
13(1)	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.
14(1)	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
15(1)	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
16(1)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
17(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
18(1)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.
19(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
20(1)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G
21(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
22(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
23(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.
24(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
25(1)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
26(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
27(1)	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
28(1)	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
29(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
30(1)	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
31(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
32(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33(1)	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
34(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table A2. Continued.

	Nucleotide position																	
Haplotype	277	289	313	379	391	406	493	496	601	658	715	769	772	802	820	841	856	861
35(1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
36(1)	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.

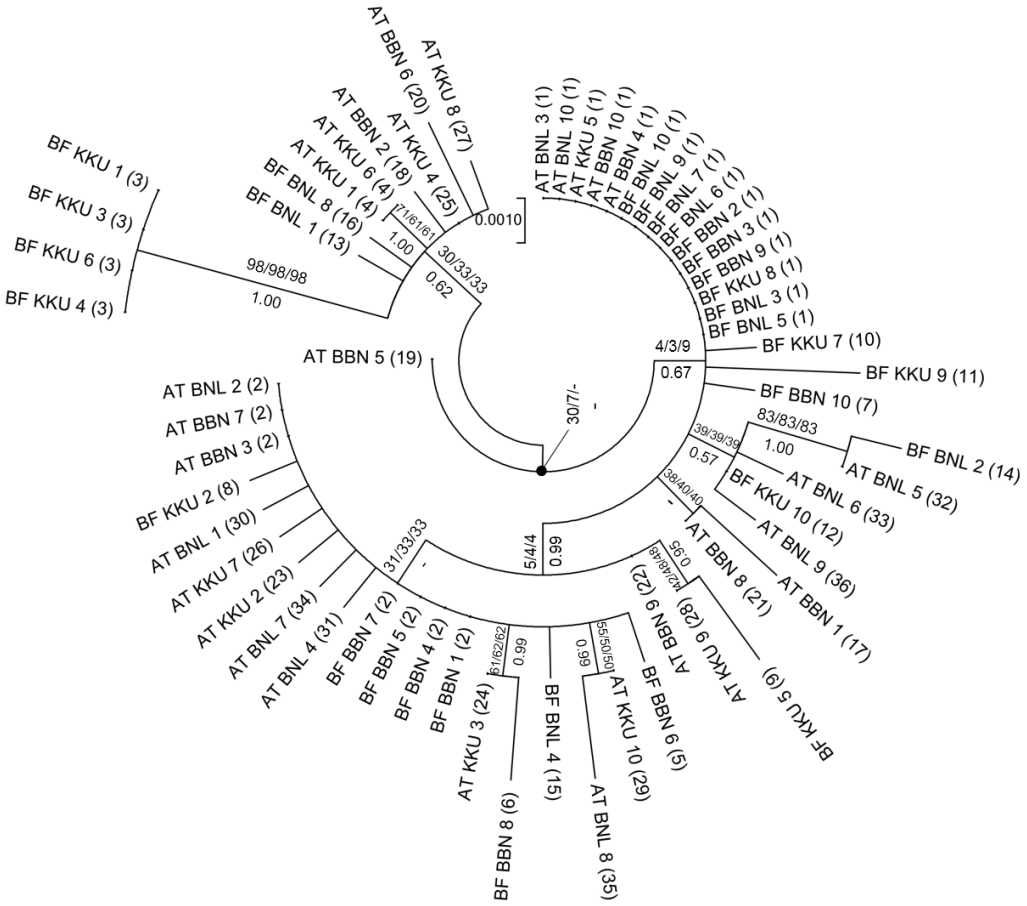

Figure A1. The maximum likelihood tree of 60 individuals of C. pomona in Khon Kaen Province based on the Tamura-Nei model (Tamura & Nei, 1993). The haplotype number is given in parentheses. The numbers above the tree branches show 3 values of bootstrap supports from Maximum likelihood/Neighbor joining/Maximum parsimony method, respectively, and the numbers below the tree branches show Bayesian inference support.

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