Genetic Evolution of Asian Predatory Wasp, *Vespa velutina*, in Northern of Thailand Based on Cytochrome Oxidase Subunit I DNA Barcoding

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ABSTRACT

The Asian predatory wasp, *Vespa velutina* was found in northern Thailand. Due to the environmental changed which might influence on genetic evolution in insect, therefore, the phylogeny and genetic evolution of this *V. velutina* is required. This study aimed to investigate the genetic evolution of *V. velutina* using cytochrome oxidase subunit I (COI) as DNA barcoding comparing to the other species of *Vespa* spp. The %GC-content of *V. velutina*'s COI in this study is 33.78% which is higher than that of an average in *Vespa* group (30.64±1.49%). The Maximum Likelihood tree could categorize *Vespa* spp. into 4 groups and 9 monophyly. The specimen in northern Thailand was separated from the other *V. velutina* which was revealed by the very high genetic distance (K2P) of 0.1712. These results can conclude that COI could be used as the powerful DNA barcode for *Vespa* spp., and COI nucleotide of *V. velutina* in each locality has highly differenced in genetic evolution which *V. velutina* in northern Thailand has the highest genetic evolution which mean genetic evolution depends on environmental influence. Moreover, this research could suggest that the *V. velutina* sampled from northern of Thailand might be another subspecies of *V. velutina*.

Keywords: COI, Thailand, Vespa, Phylogeny

INTRODUCTION

The Asian predatory wasp, *V. velutina* is native in South East Asia (Villemant et al., 2006) which widespread in Asia, from India to China and as far as Indonesia (Archer, 1994) which was recorded in the following regions: East Asia; China including Hong Kong, Japan, South Korea, and Taiwan, Southern Asia; Afghanistan, Bhutan, India, Nepal, Pakistan, South East Asia; Indonesia, Laos, Malaysia, Myanmar, Vietnam including Thailand (Archer, 2012). After 2000, this wasp has spread to Europe from southwest of France (Haxaire et al., 2006; Villemant et al., 2006a,b, Rome, 2019) to nearby countries following; Spain (López et al., 2011), Portugal (Grosso-Silva and Maia, 2012), Belgium (Rome et al., 2013), Italy (Demichelis et al., 2014), UK (Keeling et al., 2017), Netherlands (Smit et al., 2018), the Balearic Islands (Leza et al., 2018) and Germany (Witt, 2015). *V. velutina* is

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considered as an enemy of honey bees which caused in colony death (Qun, 2001; Tan et al., 2005 and 2007) in some parts of Asia similar to the study in Europe (Monceau et al., 2014). Besides, *V. velutina* also eat the ripe fruit such as apples, plums, grapes, etc. and cause damage in orchards during the fall (Villemant et al., 2010). Nevertheless, this wasp act as a biological pest control agent in which they prey spectrum of insects that consisted of 59% hymenopterans, 32% dipterans, and 9% other insects depend on the different environment varieties (Matsuura and Yamane, 1990). The characteristic of *V. velutina* could be recognized by an entirely dark brown-black body with a thin yellow stripe on the dorsal border of the first abdominal tergite, an orange-yellow band towards the end of the fourth abdomen tergite, and yellow legs. However, *V. velutina* is highly variable in color and 10 subspecies have been identified (Vecht, 1957; Carpenter and Kojima, 1997; Nguyen et al., 2006).

Cytochrome oxidase subunit I (COI) gene is one of the favors of molecular markers for animal identification depends on its unique property, high precision, and variation. COI is the catalytic subunit of the enzyme which is predominantly embedded in the membrane of the mitochondrial crista. COI was used as a DNA barcode for identification in various animals including insects such as fireflies and pets (Hebert et al., 2003; Doyle and Gaut, 2000; Jusoh et al., 2014; Kumar et al., 2015). The divergence of COI was estimated in five families of moths and caterpillars; Arctiidae, Geometridae, Noctuidae, Notodontidae, and Sphingidae, which revealed as different levels for within-family, within-genus and within-species as 11.16%, 6.84%, and0.25%, respectively (Waugh, 2018). Interestingly, COI has powerful to identify the six species of extinct birds in New Zealand when using 2.7% divergent for COI barcode and can improve the power of identification with decreasing of divergent percentage (Waugh, 2018).

COI was used as a powerful tool for molecular identification in *Vespidae*. There are many reports succeed to identify and construct the phylogeny relationship of these insects such as *V. velutina* in South Korea (Namin and Jung, 2020), Egyptian wasps (Abd-El-Samie et al., 2018), *Vespula* and *Dolichovespula* (Lopez-Osorio et al., 2014), *Polistella* in Vietnam (Nguyen et al., 2018), Nocturnal honest, *Provespa*, in Japan (Saito and Kojima, 2011), *Stenogastrinae* (Huang et al., 2019) and *Provespa* (Persson, 2015). In South Korea, *V. velutina* from different localities has a strong relationship based on COI (Namin and Jung, 2020). Likewise, Egyptian wasps; *V. orientalis* Linnaeus, *Polistes bucharensis* Erichson, and *P. mongolicus* du Buysson in different localities revealing unrestricted gene flow between them (Abd-El-Samie et al., 2018). In contrast, *Paravespula flaviceps* specimen from China diverges to that Japan specimen (Persson, 2015). Besides, the *V. velutina* from two distant sources of the United Kingdom (UK) and Japan was matched into the difference haplotype (Takahashi et al., 2018). The similar report was found in *Apis cerana* which showed the different genetic structure in the different localities of Japan depended on environmental influence. Besides that, the genetic structure of *A. cerana* is as same as those of Korea and Russia but different from those of Taiwan (Nagamitsu et al., 2016) and it also occurred in birds (Liu et al., 2013). This indicated that the genetic variation affected by environmental influence. Species richness of insects depends on the environment such as geology, climate, and habitat. The forest in northern of Thailand

is highly geography structure which influences on species diversity (Finnamore, 1997; Pianka, 2000; Araújo et al., 2006). Six *Vespa* species were found in northern of Thailand including *V. velutina* (Jongjitvimol and Urtgam, 2019).

Now a day, northern of Thailand's forest was destroyed and caused in ecological changed. This issue might be affected as reducing of insect species richness including the wasp (LaSalle and Gauld, 1997) and could provoke the genetic changed in *Vespa* spp. Therefore, phylogeny and genetic evolution will help to clarify the environmental influence of this *V. velutina*. In order to understand the genetic evolution of *V. velutina* found in northern of Thailand, this study investigates the COI sequence of *V. velutina* and constructs a phylogenetic tree with the wasp in the genus *Vespa*. This information will be helpful for further analysis of *Vespa* spp. genetic evolution.

MATERIALS AND METHODS

Sample collection and morphological study

The colony of Asian predatory wasp, *V. velutina*, was collected from forest in northern of Thailand. Adult wasp was classified using their morphology according to Jongjitvimol (2008) whereas larvae were kept at 4°C prior to DNA extraction.

Genomic DNA extraction

Genomic DNA was extracted from each of 30 samples in the single colony using 50-100 mg of muscle tissue using BioFact™ Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) according to the manufacturers' instructions. The extracted DNA was analyzed in 1%agarose gel electrophoresis containing 1xSYBR® Safe DNA gel stain (Invitrogen, USA) and kept at -20°C for further analysis.

Cytochrome oxidase subunit I (COI) geneamplification

Cytochrome oxidase subunit I(COI) gene was amplified using Polymerase chain reaction (PCR) (Mullis et al., 1986). Twenty ng of total DNA was used in PCR amplification with *Taq* DNA polymerase (Invitrogen, USA) according to the manufacturers' instructions. The PCR master mix of 25 µl containing 1x *Taq* buffer, 3 mM $MgCl_2$, 200 µM of each dNTPs, 200 nM of each universal COI primer (C1-J-1751 (alias Ron)-forward; 5'-GGAGCTCCTGACATAGCATTCCC-3' and C1-J-2191 (alias Nancy) - reverse; 5'-CCCGGTAAAATTAAAATATAAACTTC-3') (Simon et al., 1994) was carried out in T100™ Thermal Cycler (Bio-Rad, USA) with optimal condition following; initiated at 94°C for 3 min and amplified by 30 cycles of 94°C for 45 seconds, 47.5°C for 30 seconds and 72°C for 1 min followed by incubating at 72°C for 7 min for a final extension. The PCR products were analyzed on 1.5% agarose gel electrophoresis containing 1xSYBR® Safe DNA gel stain (Invitrogen, USA) and visualized under the UV light with Bio-Rad Gel Documentary (Bio-Rad, USA).

Nucleotide sequencing and analysis

PCR product was purified using BioFact™ Gel & PCR purification System (BIOFACT, Daejeon, Korea) according to the manufacturers' instructions. The DNA was eluted with 30 µl of Elution buffer. The purified PCR product was sequenced via Sanger DNA sequencing by Bionics Company (Korea). The nucleotide sequences

were aligned with ClustalW, Pairwise genetic distances were estimated using Kimura two-parameter model (K2P) and a phylogenetic tree was constructed using Cytochrome oxidase subunit I nucleotide with Maximum Likelihood method and Bootstrap 1000 in MEGAX (Kumar et al., 2018).The pairwise COI sequences was performed with 17 COI sequences of 15 *Vespa* species (Table 1) and *P. olivaceus* was used as an out-group. COI sequences were collected from GenBank (NCBI). The GC content is the percentage of nitrogenous bases on the DNA molecule which are either guanine (G) or cytosine (C). The %GC content is calculated as [Count(G + C)/Count(A + T + G + C)] x 100% by using MEGA X (Kumar et al., 2018).

RESULTSAND DISCUSSION

Morphological study of *V. velutina*

Adult *V. velutina* is about 2 cm in a total length, bright brown head with dark brown compound eyes. Body consisted of the black abdomen whiles the first and second abdominal segments are dark brown. The orange-brown band was observed in posterior margin of the first and second abdominal segments. The middles of the third to sixth gasteral segments are dark orange with black marking. The last gasteral segment appeared as orange brown (Figure 1). Although the morphological data can be used for specific identification of the wasp examined, it is the same as species found elsewhere in around world (Barthélémy, 2008; Liu et al., 2015; Ueno, 2015). Therefore, the morphology and the other characters of this sample should be thoroughly identify and compare to the other *V. velutina*.

Figure 1 Morphology of Asian predatory wasp, *V. velutina*.

Nucleotide composition in COI of *V. velutina*

The genomic DNA of *V. velutina* was used for PCR amplification using a specific primer to the COI gene which provides PCR product about 500 bp in agarose gel. Thirty PCR products of COI were sequencing which obtains the partial sequence of COI as 490 bp in length. The similarity of COI in all of those 30 COI sequences in single colony was found as 100%. This result interpreted that there is no divergence of COI with in the colony. This result contrast to the COI data in Korean bumble bees which closely related to the continental populations than to allopatric population and could be distinguished into the haplotype (Han et al., 2019). The COI nucleotide sequence was submitted to GenBank and subjected to homology matching in NCBI Blastn. The highest identical sequence (87.37%) revealed this isolate as *V. velutina* (AP018461.1) which was collected from the UK which sister to the French haplotype (Takahashi et al., 2018). Moreover, the invading of *V. velutina* in Japan was categorized into the different group with *V. velutina* in the UK which is matched to the Korean haplotype (Takahashi et al., 2018), therefore, *V. velutina* in Japan might be difference originated from *V. velutina* in northern of Thailand.

The Cytochrome oxidase subunit I sequence of *V. velutina* was aligned together with the other 17 of *Vespa* COI sequences (Table 1) in ClustalW (MEGAX). The nucleotide frequency of Cytochrome oxidase subunit I was calculated and presented as an average of %GC-content as 30.64±1.49 (Table 1) which the highest is *V. velutina* (in this research) following by *V. affinis* (KJ147242.1) as 33.78% and 32.44%, respectively whereas %GC-content in the other 2 *V. velutina* were found as only 28.72% (Table 1). This result suggested that genetic evolution of *V. velutina* in northern of Thailand might be different from the other *V. velutina* according to the important role of GC content on genomic architecture and genetic evolution in plant (Smarda et al., 2014).

Phylogenetic tree construction of *V. velutina* using COI nucleotides

The Maximum Likelihood (ML) tree was performed based on COI sequence with 1000 replicate bootstrap method. All of 18 COI sequences of *Vespa* were used for phylogenetic tree construction whereas *Polistes olivaceus* (KY836116.1) was used as an out-group. Four groups of *Vespa spp*. were categorized via ML tree comprise of (1) *V. velutina* (AP018461.1) and *V. velutina* (KY224073.1), (2) *V. simillima* (KF933080.1) and *V. bicolor* (KF933079.1), (3) *V. soror* (KF933086.1) and *V. mandarinia* (KF933085.1) and (4) *V. ducalis* (KF933084.1), *V. tropica* (KM455116.1), and *V. tropica* (MN893829.1) while the other 9 specimen appeared as monophyly (Figure 2). The categories in this *Vespa* spp. phylogenetic tree supported the morphology-based phylogeny of *Vespa* spp. (Archer, 1994). This finding concluded that COI could be used as a powerful molecular marker for *Vespa* spp. identification according to those in the other Vespidae (Abd-El-Samie et al., 2018; Huang et al., 2019; Lopez-Osorio et al., 2014; Namin and Jung, 2020; Nguyen et al., 2018; Persson, 2015; Saito and Kohima, 2011). *V. analis* (AB585948.1) and *V. luctosa* (KY659794.1) have closely relationship with three *V. velutina* according to the characteristic study (Archer, 1994). Although, the bootstrap confidence at the node is only 3%, the clade was rejected (Soltis & Soltis, 2003). In this *Vespa* spp.

phylogenetic tree, *V. velutina* (in this study) was categorized into a monophyly which contrast to the within-Korean *V. velutina* phylogeny. *V. velutina* from different localities of Korea was clustered together with 100% support which suggested that the different localities in Korea not far enough for restricted of genetics transfer (Namin and Jung, 2020).

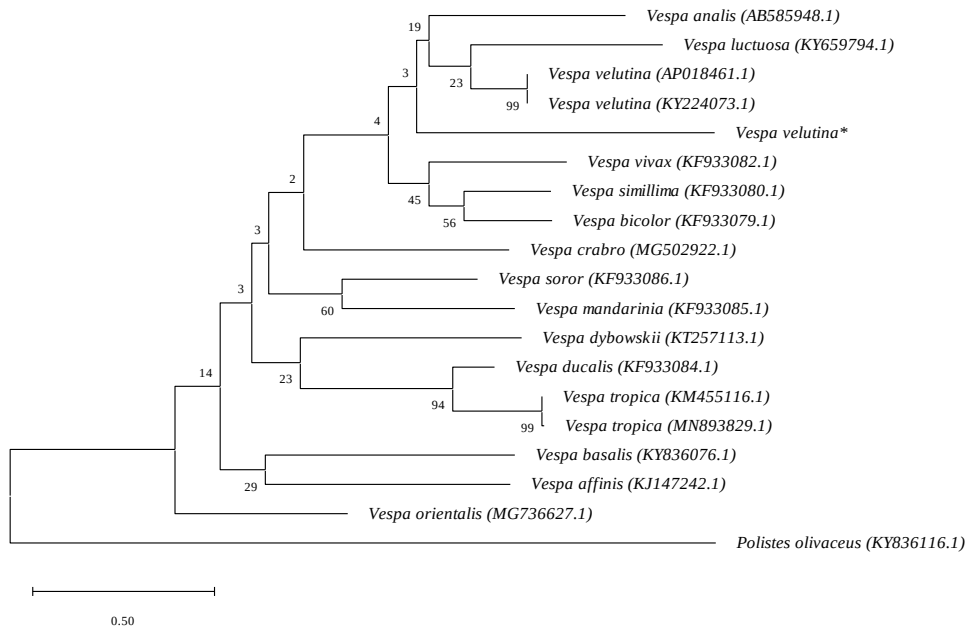
In this study *V. velutina* specimen was compared to *V. velutina* (AP018461.1) (Takahashi et al., 2018) and *V. velutina* (KY224073.1) which collected from the UK. Therefore, this study might be presented as the very far of genetic divergent similar to the comparison in *V. velutina* in Iki Island Japan and Jersey Island, the UK which matches to the different populations as Korean and French *V. velutina* (Takahashi et al., 2018). This finding supports the localities' influence on the genetic evolution of *V. velutina*.

Table 1 Nucleotide frequency of Cytochrome oxidase subunit I in *Vespa* spp.

Species	%GC-content	Specimen source	Reference
V. velutina	33.78	Northern of Thailand	In this research
V. velutina (AP018461.1)	28.72	Jersey Island, the UK	Takahashi, 2018
V. velutina (KY224073.1)	28.72	The UK	Budge, unpublished
V. tropica (KM455116.1)	31.12	India	Kavitha, unpublished
V. tropica (MN893829.1)	31.65	India	Balachandar, unpublished
V. crabro (MG502922.1)	30.58	Canada	Dewaard, unpublished
V. basalis (KY836076.1)	30.32	Pakistan	Ashfaq, unpublished
V. orientalis (MG736627.1)	30.58	Egypt	Abd-El-Samie, 2018
V. luctuosa (KY659794.1)	28.45	Philippines	Fontanilla, unpublished
V. soror (KF933086.1)	31.38	USA	Perrard, 2013
V. mandarinia (KF933085.1)	31.38	USA	Perrard, 2013
V. ducalis (KF933084.1)	31.92	USA	Perrard, 2013
V. vivax (KF933082.1)	30.58	USA	Perrard, 2013
V. simillima (KF933080.1)	31.39	USA	Perrard, 2013

V. bicolor (KF933079.1)	30.85	USA	Perrard, 2013
V. analis (AB585948.1)	29.79	South East Asia	Saito, 2011
V. dybowskii (KT257113.1)	27.92	USA	Lopez-Osorio, 2015
V. affinis (KJ147242.1)	32.44	USA	Lopez-Osorio, 2014
Avg. ± SD	30.64 ± 1.49		

Although, *V. velutina* sample in northern of Thailand shows the highest evolution among the *Vespa* spp. and was separated to the other *V. velutina* (Figure 2). Whereas, evolutionary divergences of this specimen still closer to those two *V. velutina* (AP018461.1) and *V. velutina* (KY224073.1) with a higher genetic distance of 0.1712 (Table 2) than the others. *V. velutina* specimens using in this study has 17.12% genetic divergent compare to the same species which is higher than that of species levels (Waugh, 2018). The result suggested that *V. velutina* sampled in northern of Thailand might be the subspecies of *V. velutina* which is the same haplotype with the UK and French *V. velutina*. This result correlated to a previous study which found that *V. velutina* could be identified as at least 10 subspecies spread in various localities (Vecht, 1957; Carpenter and Kojima, 1997; Nguyen et al., 2006).



Note:* the wasp was found in northern of Thailand.

Figure 2 Phylogenetic tree of Cytochrome oxidase subunit I sequence from *V. velutina* using Maximum Likelihood model comparing with the other

Vespa spp. The nodes represent bootstrap values; *P. olivaceus* was used as an out-group (Tamura and Nei,1993; Kumar et al., 2018).

Table 2 Evolutionary divergence between sequences estimated with Kimura-two parameter (Tamura et al., 2004; Kumar et al., 2018)

Species	*K2P of V. velutina* (in this study) to the others
V. velutina (AP018461.1)	0.1712
V. velutina (KY224073.1)	0.1712
V. crabro (MG502922.1)	0.1817
V. simillima (KF933080.1)	0.1901
V. vivax (KF933082.1)	0.1902
V. mandarinia (KF933085.1)	0.1969
V. analis (AB585948.1)	0.1979
V. basalis (KY836076.1)	0.2022
V. luctuosa (KY659794.1)	0.2043
V. dybowskii (KT257113.1)	0.2051
V. soror (KF933086.1)	0.2106
V. bicolor (KF933079.1)	0.2118
V. ducalis (KF933084.1)	0.2180
V. orientalis (MG736627.1)	0.2204
V. affinis (KJ147242.1)	0.2245
V. tropica (KM455116.1)	0.2279
V. tropica (MN893829.1)	0.2279
Polistes olivaceus (KY836116.1)	0.2606

CONCLUSIONS

This study could conclude that 1) COI is the effective DNA marker for *Vespa* species identification; however, it could not be used for the genetic diversity study within the colony because it is located in the mitochondrial genome. Therefore, the nuclear gene such as *ITS2* will help to improve colony genetic diversity study. 2) *V. velutina* in northern of Thailand might be the subspecies of *V. velutina* due to the homology of COI is low as only 87.37% which is might be originated from different localities to East Asia; Korean and Japan *V. velutina* population. 3) The genetic evolution of *V. velutina* in northern Thailand is higher than those of the other *Vespa* spp. with the highest %GC-content. Finally, 4) this research supports that the genetic evolution of *V. velutina* depends on the environmental influence. For further study, the phylogenetic tree of *V. velutina* should be constructed using COI nucleotide sequence data from various localities to understand the history and genetic evolution including invasion of this species.

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