J. Entomol. Res. Soc., 22(3): 227-237, 2020
Print ISSN:1302-0250
Research Article
Online ISSN:2651-3579

A Bioinformatics Study to Detect the Genetic Characteristics of *Vespa* Hornets (Hymenoptera: Vespidae)

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ABSTRACT

Hornets especially from genus *Vespa* can attack honey bee colonies, causing economic damages to beekeeping. Also, these hornets can cause damages to some crops. Basically, *Vespa* hornets occur in different parts of the world including Asia, North Africa, and Europe. Recently, the hornet, *Vespa velutina*, has invaded Europe and considered as a serious pest to beekeeping. This study aimed to investigate the genetic similarities between five *Vespa* hornets: *V. orientalis*, *V. ducalis*, *V. mandarinia*, *V. affinis*, and *V. velutina* depending on analyzing the sequences of mtDNA utilizing bioinformatics. The phylogenetic relationships, segments from the enzymatic digestion, open reading frames, and number of shared gene cluster families were investigated to detect the genetic similarities. Also, specific primers to discriminate between these hornets were designed and tested in silico. The potential infection of these hornets with similar pathogens was emphasized in light of the obtained results.

Key words: Honey bees, invasion, predators, genes, bioinformatics.

Abou-Shaara, H.F. & Elbanoby, M.I. (2020). A bioinformatics study to detect the genetic characteristics of *Vespa* hornets (Hymenoptera: Vespidae). *Journal of the Entomological Research Society*, 22(3), 227-237.

INTRODUCTION

Hornets belong to *Vespa* (Hymenoptera, Vespoidea, Vespidae) are considered as pests to honey bees and may cause damages to some crops. There are about 22 *Vespa* hornets (Archer, 2012), and these hornets are differed in their geographical distribution. The oriental hornets, *Vespa orientalis* Linnaeus, 1771, are existed in some Asian countries, North Africa, and some European countries, and accidently found in Mexico (Dvořák, 2006). Some hornet species distribute in Asia, e.g. Japan, China, Korea, including; the black-tailed hornet *Vespa ducalis* Smith, 1852 (predator to immature stages of paper wasp), the giant hornet *Vespa mandarinia* Smith, 1852 (a serious pest to agriculture in Japan, Matsuura & Sakagami, 1973), the lesser banded hornet *Vespa affinis* Linnaeus, 1764 (predator to wasps and bees), and the Asian hornet *Vespa velutina* Lepeletier, 1836 (predator to honey bees, Tan et al, 2007). Recently, *V. velutina* was recorded in Iberian Peninsula (López, González, & Goldarazena, 2011) and has invaded Europe (Grosso-Silva & Maia, 2012; Monceau, Bonnard, & Thiéry, 2014).

Previous studies on the oriental hornets have focused on many aspects including behavioral aspects and nest building (Ishay, 1976; Kugler, Motro, & Ishay, 1979; Ishay et al, 1995), body properties and pheromones (Ishay, Ikan, & Bergmann, 1965; Ikan, Gottlieb, Bergmann, & Ishay, 1969; Raina & Singh, 1996), venom components (Edery, Ishay, Lass, & Gitter, 1972; Rosenberg, Ishay, & Gitter, 1977; Schoeters & Billen, 1995; Haim, Rimon, Ishay, & Rimon, 1999). Some studies have focused on their damages to honey bee colonies (Taha, 2014) or crops (Al-Mahdawi & Al-Kinani, 2011), and potential control strategies (Bacandritsos, Papanastasiou, Saitanis, & Roinioti, 2006; Mahdi, Glaiim, & Ibrahim, 2008) including possible biological trends (Haddad, Fuchs, Haddaden, & Kopelke, 2005) and honey bee response (Papachristoforou et al, 2008; Papachristoforou, Rortais, Sueur, & Arnold, 2011). Studies on other *Vespa* hornets have concentrated on similar aspects, for examples mating behavior of *Vespa ducalis* (Takahashi, Akimoto, Hasegawa, & Nakamura, 2002), Venom of *Vespa affinis* (Sukprasert et al, 2013), behaviors and control of *Vespa velutina* (Abrol, 1994), and response of bees to *Vespa mandarinia* attack (Sugahara & Sakamoto, 2009).

The mtDNA of some *Vespa* species have been sequenced including: *V. mandarinia* (Chen, Wei, & Liu, 2016), *V. orientalis* (Haddad et al, 2017), *V. affinis* (Okuyama, Martin, & Takahashi, 2017), *V. ducalis* (Kim, Jeong, Jeong, Kim, & Kim, 2017), and *V. velutina* (Takahashi, Okuyama, Minoshima, & Takahashi, 2018). The phylogenetic relationships between *Vespa* have been studied based on morphological characters (Perrard, Pickett, Villemant, Kojima, & Carpenter, 2013) while few studies have focused on the genetic characteristics of these hornets. Indeed, bioinformatics can be effectively used to investigate the genetic relationships between organisms (Abou-Shaara & Bayoumi, 2019). Therefore, this study aimed to investigate the genetic relationships between some *Vespa* hornets based on the mtDNA sequence with comparing genetic characteristics. This study expands the knowledge about genetic characteristics of *Vespa* hornets.

METHODS

Sequences of *Vespa* hornets

The mtDNA sequences were downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov) and utilized in this study. The sequences were to *Vespa orientalis* (NCBI Reference Sequence: NC_034003.1, 16101 bp), *Vespa ducalis* (GenBank: KX950825.1, 15779 bp), *Vespa affinis* (NCBI Reference Sequence: NC_039134.1, 19109 bp), *Vespa velutina* (GenBank: AP017943.2, 16416 bp), and *Vespa mandarinia* (NCBI Reference Sequence: NC_027172.1, 15902 bp).

Phylogenetic tree

The sequences were aligned using ClustalW, then maximum likelihood method and the Jukes-Cantor model (Jukes & Cantor, 1969) was used to construct the phylogenetic tree. This analysis was performed using MEGA7 (Kumar, Stecher, & Tamura, 2016).

Digest

The available restriction enzymes at Genome Compiler 2.2.88 (http://www.genomecompiler.com) were used to digest the full sequences of *Vespa* hornets, and then simulated on gel using NEB 100bp ladder. The resulted fragments were compared to detect similarity aspects according to Abou-Shaara (2019a; 2019b).

Open reading frames

For each *Vespa* species, open reading frames (ORFs) were identified using Genome Compiler 2.2.88 with start codons of ATG or GTG or TTG or CTG and minimum length of amino acids of 100, considering all frame types. The identified ORFs were compared between studied hornets to check the similarities.

Shared gene cluster

The number of shared gene clusters between the studied hornets was identified using OrthoVenn (http://www.bioinfogenome.net) utilizing proteins downloaded from Uniprot according to Abou-Shaara (2019a; 2019b).

Primers to discriminate between *Vespa* hornets

Primers were designed using Genome Compiler 2.2.88 utilizing the option of auto primer design. Primers were designed to amplify the region between 3000 to 3100 nt. The designed primers were tested using PCR simulation of SnapGene 4.2.6 software (from GSL Biotech; available at snapgene.com) to ensure the amplification of the target sequences.

RESULTS AND DISCUSSION

Phylogenetic tree

The phylogenetic tree (Fig. 1) shows the close relationships between *V. velutina, V. mandarinia* and *V. ducalis* while no close relationship was detected between *V.*

orientalis and *V. affinis*. Previous studies support these findings, the phylogenetic analysis based on 45 morphological characters and molecular analysis showed the presence of close relationships between *V. mandarinia* and *V. ducalis* while the other three *Vespa* species were placed in different groups (Perrard et al, 2013). Based on the nucleotide sequences of the 13 protein-coding genes, close relationships between *V. mandarinia*, *V. ducalis* and *V. affinis*, were found (Okuyama et al, 2017; Takahashi et al, 2018), and between *V. mandarinia* and *V. ducalis* (Kim et al, 2017; Zhang, Huang, Chen & Li, 2018). On the contrary with the current study, a close relationship was found between *V. mandarinia* and *V. orientalis* based on the un-gapped sequences of a multiple sequence alignment (Haddad et al, 2017), while *V. orientalis* and *V. velutina* were placed in close groups based on the nucleotide sequences of the 13 protein-coding genes (Takahashi et al, 2018). The present and previous studies confirm the high relatedness between *V. mandarinia*, and *V. ducalis* while the relationships between other *Vespa* species showed variation in relation to the analysis method. It is clear that the analysis method can result in different phylogenetic relationships between *Vespa* hornets.

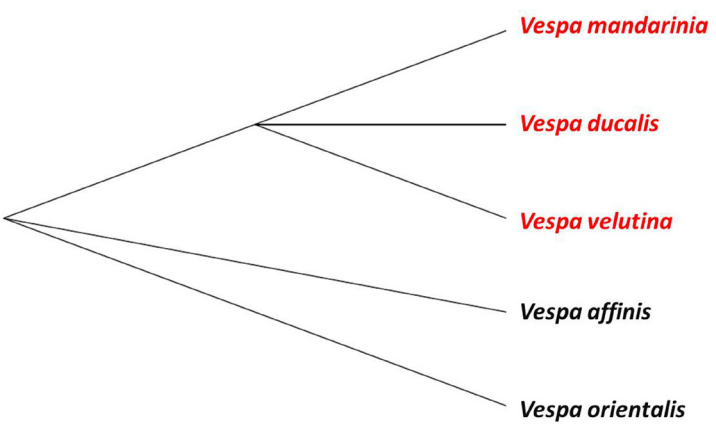

Fig. 1. The phylogenetic relationships between *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

Digest

The fragments resulted from the enzymatic digestion of the mtDNA sequences of *Vespa* hornets are shown in Fig. 2. The number of fragments was 15, 14, 17, 16, and 21 for *V. orientalis, V. ducalis, V. affinis, V. velutina* and *V. mandarinia*, respectively. The number of similar fragments between *V. mandarinia* and the other hornets was 6, 4, 6, and 9 fragments with *V. velutina, V. affinis, V. ducalis,* and *V. orientalis,* respectively. The closely related species *V. mandarinia, V. velutina,* and *V. ducalis* as shown from the phylogenetic tree had the same number of similar fragments. This supports the genetic relationships between these three hornets. In general, the presence of similar fragments among *Vespa* hornets is anticipated because they belong to the same family and genus.

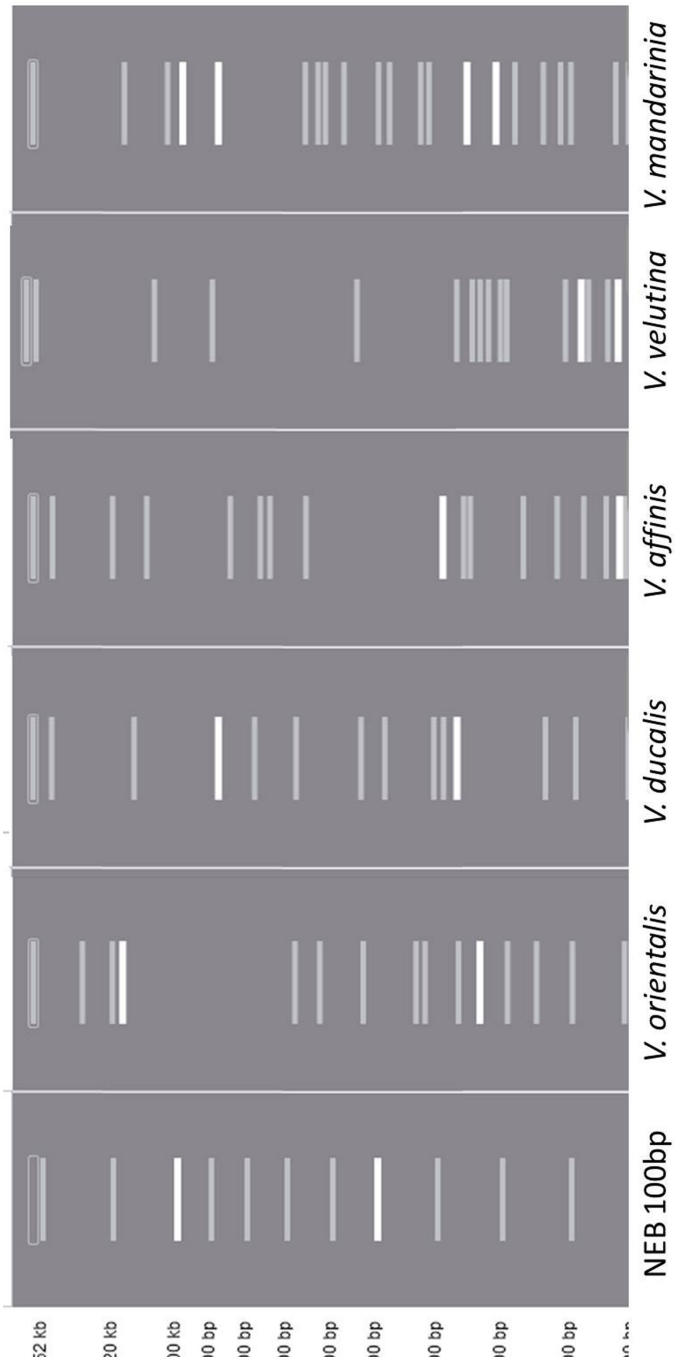

Fig. 2. Gel simulation to the resulted fragments from the enzymatic digestion of mtDNA sequences of *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia* using Genome Compiler.

Open reading frames

The detected ORFs were somewhat similar between *V. mandarinia, V. velutina,* and *V. ducalis* unlike *V. orientalis* and *V. affinis* (Fig. 3). This finding supports the phylogenetic relationships detected based on the phylogenetic tree, and suggests the low number of ORFs with 100 amino acids in the studied hornets.

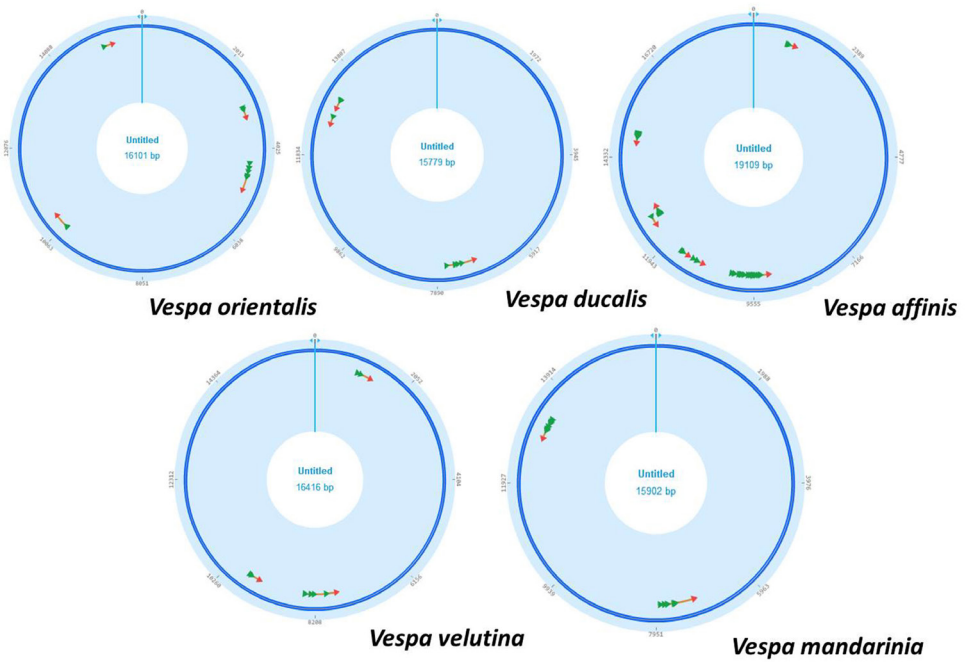

Fig. 3. Open reading frames using Genome Compiler for *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

Functional analysis

The shared gene cluster families were 491 among all the five hornets (Fig. 4), and this because all hornets belong to the same family and genus. Indeed, *V. mandarinia, V. velutina,* and *V. ducalis* shared 112 gene cluster families (Fig. 4). This supports their close relationships than the other two hornet species. Few shared gene cluster families (<38) were detected between *V. orientalis* and *V. affinis* and the other three hornet species, suggesting lacking of high genetic similarities.

Primers to discriminate between *Vespa* hornets

Specific primers were designed to each hornet (Table 1), and tested against amplified region (Fig. 5). These primers can clearly discriminate between these five species, as each primer can give bands only with the target species while no bands can be obtained with the non-target species.

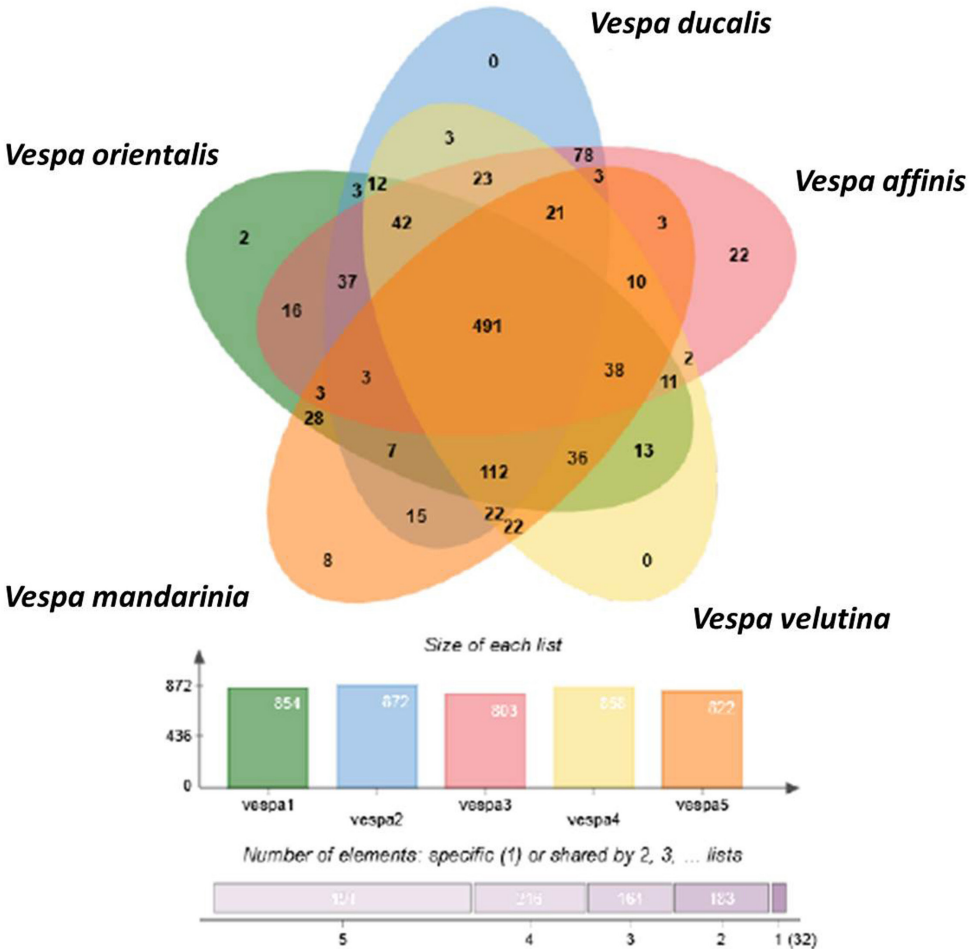

Fig. 4. Gene clusters produced to *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

CONCLUSION

The study confirms the close genetic relationships between three *Vespa* species: *V. velutina, V. mandarinia* and *V. ducalis* utilizing different analytical techniques. Due to the high genetic similarities detected in this study, it is anticipated that these hornets may be infected with similar pathogens and can host similar pathogens, points which worth further investigations. Especially, some viruses including Israeli acute paralysis virus, deformed wing virus and Moku virus were detected in *Vespa* hornets (Yañez, Zheng, Hu, Neumann, & Dietemann, 2012; Garigliany et al, 2017; Forzan, Sagona, Mazzei, & Felicioli, 2017). Also, the study presented specific primers to discriminate between the five *Vespa* species: *V. orientalis*, *V. ducalis*, *V. affinis*, *V. velutina*, and *V. mandarinia.*

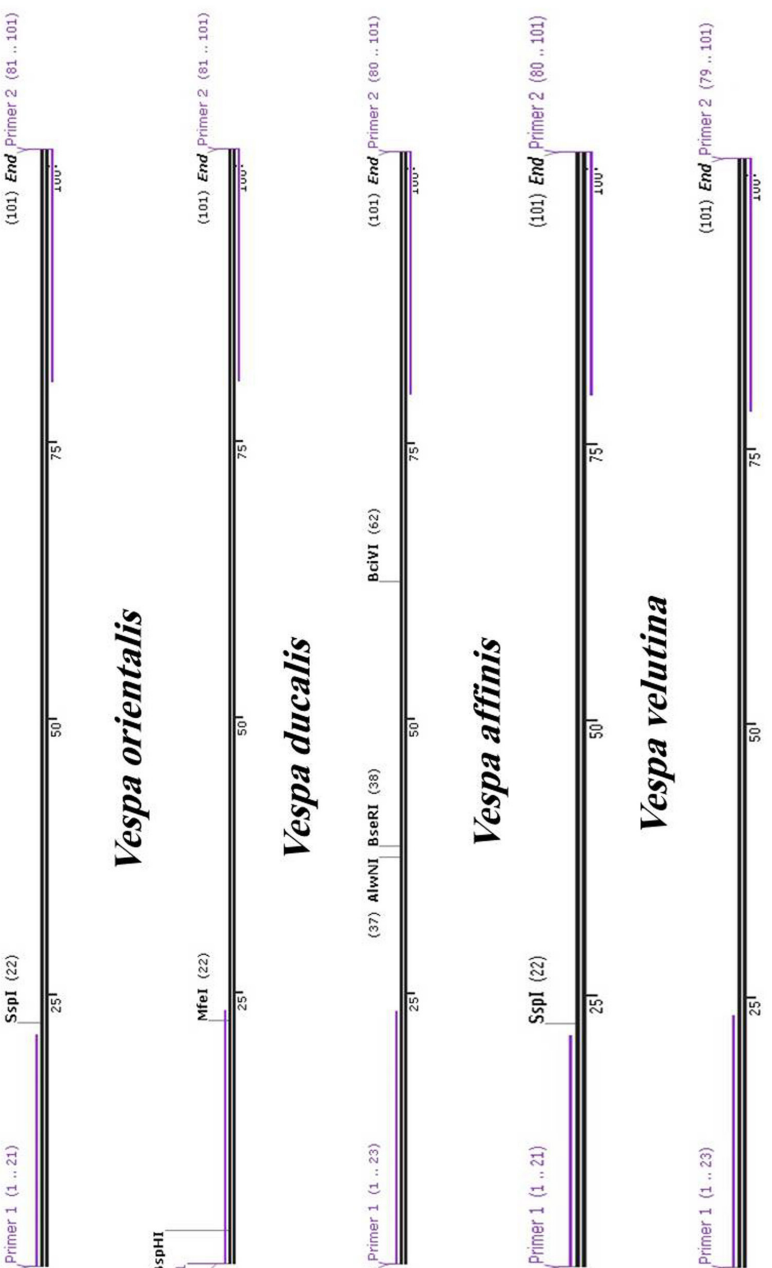

Fig.5. PCR simulation for the amplified regions using the specific primers to *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia* using SnapGene.

Table 1. Specific primers to identify *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia.*

Species	Type	Sequences
Vespa orientalis	Forward 5’	TGAAATTAGGTAGGTATGGAA
	Reverse 5’	TCCTCCTACAATTCCTAATGT
Vespa ducalis	Forward 5’	CTTCATGAAATGTTATCTCATCA
	Reverse 5’	AATTCGTTGTGATATAAATGA
Vespa affinis	Forward 5’	TTAATTACAAGAAACTTTATCGG
	Reverse 5’	AAATCAACGGCAGGAGAATTGT
Vespa velutina	Forward 5’	TAAAAATTCAATTTACTTCAA
	Reverse 5’	GTAATCTGAGTAACGTCGTGGG
Vespa mandarinia	Forward 5’	CGAATTATCTTATATAAATTTTT
	Reverse 5’	TTAGTAGTATAGGGAATTTCTTG

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Received: August 09, 2019 *Accepted: September 22, 2020*