Original Article

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Morphometric and Genetic Variation of Tropilaelaps Mites Infesting *Apis dorsata* and *A. mellifera* in Thailand

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Abstract

The majority parasitic bee mites of Thailand in genus *Tropilaelaps* are infesting colonies of native bees (*Apis dorsata*) and introduced bees (*A. mellifera*). The investigation aims to study morphological and genetic variation of *Tropilaelaps* mites infected different hosts. Adult mites were collected from honey bee brood throughout Thailand. Traditional and geometrical morphometrics were measured on photograph by using TPS program. Additional, COI gene variations were examined by PCR-RFLP and nucleotides sequencing. Tree of mites relationships were constructed by NJ and MP assumptions. Morphometric results indicated *T. mercedesae* were major species infesting on *A. dorsata* and *A. mellifera*. Mophological variation represented at anal and epigynial plate, which the shape of the anal plate apex margin has been key character to identify between *T. mercedesae* (bell to blunt shape) and *T. koenigerum* (pear shape). However, the discriminant analysis suggested that geometric results were potential to classify Thai *Tropilaelaps* populations from different hosts better than traditional morphometric. Otherwise, PCR-RFLP clearly detected the site of *Dra* I and *Xba* I digestion of Thai *Tropilaelaps* morphotypes. The COI sequences of *T. koenigerum* were founded infesting only *A. dorsata* in Thailand and four sequences that related to the Thai *T. mercedesae* morphotypes. The NJ and MP tree were clearly classified Thai *Tropilaelaps* species which were suggested both from morphological and molecular analysis. This information might be basically of taxonomic status, but this should have implication for controlling these mites in Thailand and other countries.

Key words: *Apis dorsata*, COI, Geometric, Phylogeny, *Tropilaelaps*, Variation

INTRODUCTION

Honey bee mites belonging to Genus *Tropilaelaps* are serious pest of beekeeping in the world. They have tiny oval body shape and approximately 1 mm in length. *Tropilaelaps* mites are ectoparasite on honey bee body; induce of several diseases such as viruses, bacteria or protozoa destroying the bee colonies. This causes Colonies Collapse Disorder (CCD) in *Apis mellifera* beekeeping (Sammataro *et al*., 2000). The mites invade native naturally colony of giant honey bee (*Apis dorsata* or *A. larboriosa*), those are original in Asia. The first taxonomic data named *Tropilaelaps clareae*, was found from *A. dorsata* in Philippine (Delfinado-Baker and Baker, 1961). Recently *T. clareae* has shifted host to commercial honey bee (*A. mellifera*) and becomes cosmopolitan pest to destroy beekeeping. Another species was *T. koenigerum* from *A. larboriosa*, found distributing in Brunei, India,

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Nepal, Thailand and Vietnam except in Sri Lanka (Burgett *et al*., 1983; Tangjingjai *et al*., 2003). Sometimes *T. koenigerum* was found in *A. dorsata* but was not serious in *A. mellifera* beekeeping (Koeniger *et al*., 2002; Anderson and Morgan, 2007).

At present taxonomic data of the parasitic bee mites has been revised by using morphological as well as molecular study. To cite an example the mites belonging to the genus *Varroa* which infests two honey bees species i.e. *A. cerana* or *A. mellifera* can be mentioned. There are four species (*V. destructor, V. jacobsoni, V. rindereri* and *V. underwoodi*) and the serious pest of *A. mellifera* beekeeping in the world has been found *V. destructor* (Anderson and Trueman, 2000). Situation of parasitic bee mites in Thailand is worth mentioning because of their variability of genetically haplotype as genus *Varroa* infested on native host (*A. cerana*). There are 5 haplotypes as Vietnam haplotype of *V. destructer* and 4 haplotypes of *V. jacobsoni* such as Malaysia, Norththai1, Norththai 2 and Samui haplotypes (Warrit *et al*., 2006; Warrit and Lekprayoon, 2011). In Thailand, *A. mellifera* colonies were more infested with Korean haplotype of *V. destructor* than Japan/Thailand haplotype and were not found *V. jacobsoni* (Suppasat, 2007). Anderson and Morgan (2007) revised the mite belonging genus *Tropilaelaps*, naturally infested single nesting bees (*A. d. dorsata, A. d. binghami, A. d. breviligula* and *A. laboriosa*). The genus *Tropilaelaps* has two species, *T. mercedesae* and *T. thaii* among which the most predominating damage causing mite in *A. mellifera* is *T. mercedecae*. Recently, in China, the tropilaelaps mites infesting *A. mellifera* was *T. mercedesae* instead *T. clareae*, supported by using COI sequences (Luo *et al*., 2011).

Morphological variation of tropilaelaps mites showed the differences in size and shape such as ventral and dorsal plate, epigynial plate, anal plate, setae and stigmata characters. *T. clareae*, *T. koenigerum* and *T. thaii* can be identified based on the anal plate characters while *T. mercedecae* bears diverse characters (Anderson and Morgan, 2007; Anderson and Robert, 2013; Kavinseksan and Wongsiri, 2016). Morphometric analysis including traditional and geometric morphometric is powerful techniques for determining morphology (Daly, 1985; Rohlf, 1999). Recently, geometric morphometric is popular method to analyzed size, shape and orientation in several insect wings such as mosquitoes (Rohlf, 2002). Likewise, the geometric morphometric was used to investigate the mite *Aceria guerreronis* Keifer (Acari: Eriophyidae), those populations distributed in America, Africa and Asia. The geometric shape of coxigenital region (anal plate) variation of the mite is effective to show relation with their population distribution (Navia *et al*., 2006). The eleven species of oppiid mites (Acari: Oribatida) in Turkey were determined 17 measurements for traditional morphometric and were plotted 20 landmarks on ventral surface for geometric morphometric. Both of methods separated in genera of oppiid mites (Baran *et al*., 2011). Genetic variation of COI gene is popular to apply for species identification of *Varroa* and *Tropilaelaps* genera. Moreover, biogeography of mites and their native hosts were represented with the COI sequences which were hypothesized by using phylogenetic relationship. By the way, the tropilaelaps mite, examined highly fashionable identification in morphological and genetic variability of ITS and COI genes of mitochondrial DNA (Anderson and Morgan, 2007; Luo *et al*., 2011).

In Thailand, the first known species belonging to genus *Tropilaelaps* was *T. clareae*, which infested on naturally *A. dorsata* or *A. cerana* colonies. Later it also shifted and became serious pest of *A. mellifera* colonies while *T. koenigerum* infected only *A. dorsata* (Burgett *et al*., 1983; Tangjingjai *et al*., 2003). In early 2000, the *T. clareae* and *T. koenigerum* were investigated by using PCR-RFLP of ITS gene and RAPD methods to develop specific marker for identification (Tangjingjai *et al*., 2003). At present the most predominating *Tropilaelaps* mite infesting *A.*

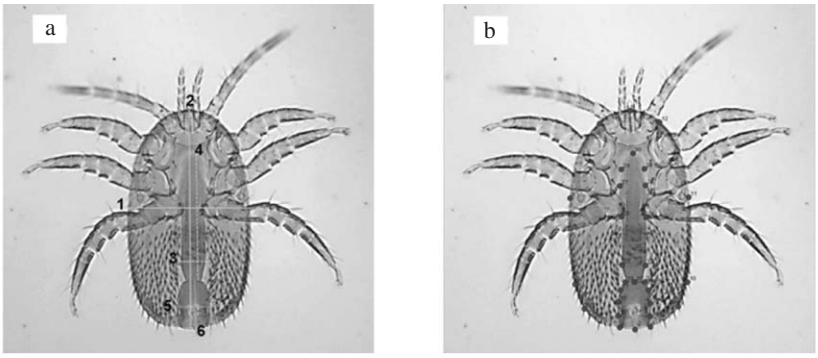

Fig. 1. Traditional morphometric was measured for 6 characters of width and length on ventral plate such as body size (-) represented 1and 2, epigynial plate (--) represented 3 and 4 and anal plate (···) represented 5 and 6 (a). Geometric landmarks were plotted 30 points on ventral side of mite image (b).

mellifera is *T. mercedesae* (Anderson and Morgan, 2007). This research aims to examine morphological and genetic variation of the tropilaelaps mites infesting native host (*A. dorsata*) and exotic host (*A. mellifera*) in Thailand. The Thai tropilaelaps mite species are identified by morphometric and COI markers.

MATERIALS AND METHODS

Thai tropilaelaps collections

The adult tropilaelaps mites were collected from honey bee brood of native host (*A. dorsata*) and exotic host (*A. mellifera*) in Thailand between 2008-2010. The mites (n=307) were collected from infesting colonies of *A. dorsata* (15 colonies, n=88) and *A. mellifera* (21 colonies, n=219). The tropilaelaps samples were kept in 70% ethanol for morphometric study and were preserved in absolute ethanol then kept them at −4°C for molecular study.

Morphometric analysis

Collected mites were mounted in Hoyer's medium (Krantz, 1978) on slide. All samples were photographed using Nikon EClipsee 200 digital microscope, and photographs were used for morphometric analysis with tpsDig 2.04 (Rohlf, 2010a). Firstly, traditional morphometric was measured width (W) and length (L) of ventral shield (Ven), anal shield (Anal) and epigynial shield (Epig) for 6 characters (W-Ven, L-Ven, W-Anal, L-Anal, W-Epig and L-Epig) in totally (Fig. 1a). Then, the statistically significance of 6 character measurements of tropilaelaps mites from different hosts were analyzed by using one-way ANOVA and discriminant analysis. Secondly, geometric morphometric digitized landmark on 152 mite photographs that divided female mite infesting *A. dorsata* for 60 samples and *A. mellifera* for 92 samples. All of tropilaelaps images were plotted 30 landmarks on ventral side by using tpsDig 2.04 (Fig. 1b). Geometric analysis calculated variation in each landmark. And, principle component analysis (PCA) were generated partial and relative warp by using tpsRelW 1.49 (Rohlf, 2010b).

Molecular analysis

DNA analysis of adult female tropilaelaps mites were investigated in mitochondrial DNA i.e. COI gene. There were 53 tropilaelaps mites, were form different hosts; *A. dorsata* (n=23) and *A. mellifera* (n=30). One mite was extracted DNA using Genelute™ Mammalian Genomic DNA Kit (Sigma, USA). Polymerase Chain Reaction (PCR) of COI was amplified using pair of primers TCF-1 and TCR-2 following Anderson and Morgan (2007). PCR product was checked on 1.5% agarose gel electrophoresis and was purified with RBC hiyield gel/PCRDNA fragments extraction kit (RBC, Taiwan). Then, PCR-RFLP

was examined with 5µl of the PCR products that were digested using *Dra* I and *Xba* I (BioLabs, UK). Restriction fragments were run on 2.0% agarose gel electrophoresis and scored restriction patterns. Further, COI sequences were generated nucleotide sequences of Thai tropilaelaps mites. Multiple sequences of Thai tropilaelaps were aligned using ClustalW in Mega 6.0 program (Tamura *et al*., 2011). All the Thai tropilaelaps haplotypes were aligned using 4 sequences of *T. clareae* (EF025464, EF025466, EF025467 and EF025468), 3 sequences of *T. koenigerum* (EF025449, EF025450 and EF025451), 9 sequences of *T. mercedesae* (EF025429, EF025430, EF025431, EF025432, EF025437, EF025438, EF025445, EF025446 and EF025447) and *T. thaii* (EF025452).

Tree reconstruction of Thai tropilaelaps mites relationship were reconstructed by using neighbor-joining (NJ) and maximum parsimony (MP) methods, additional statistical with bootstrap 1,000 replications in Mega 6.0. The first method of neighbor-joining was performed by using distance matrices with p-distance and Kimura-2 parameter (K2) models. Also maximum parsimony was generated using both models. The tree reconstruction followed Morgan and Anderson (2007) and added performance of K2 model. All of tree reconstruction methods used condense consensus tree that performed statistical value at 50% cut off following majority's rule to show relationship between Thai tropilaelaps mites and other from NCBI database.

RESULTS

Morphometric variation of Thai tropilaelaps mites

The traditional morphometric showed that almost all Thai tropilaelaps mites infesting different hosts (*A. dorsata* (Ad) and *A. mellifera* (Am)) was *T. mercedesae* (Tm), but 3 samples infesting *A. dorsata* were resembles to *T. koenigerum* (Tk) in their anal plate as pear shape. The Thai tropilaelaps mites infesting different hosts were significantly difference ($P<0.05$) in their average for 4 morphometric characters (Table 1).

Traditional morphometric results indicated that *Tropilaelaps* infesting *A. dorsata* have longer body size and anal plate than mites infesting *A. mellifera*. But, the anal plate of mites infesting *A. mellifera* was wider than the others. In contrast mites from *A. mellifera* have narrow epigynial plate. Additionally, the mahalanobis distance discriminant supported 4 morphometric characters such as length of ventral plate, length and width of anal plate and length of epigynial plate, those represented the relationship in each group of mites infesting different honey bee hosts. Moreover, the canonical discriminant function showed eigenvalue for 0.484, 100% of cumulative and canonical correlation for 0.571, also test of function showed Wilks' lambda was 0.674. And cross validation predicted group of Thai tropilaelaps mite correctly classified into each species for 78.8% (Table 2).

Table 1. Morphometric of Thai tropilaelaps mites were showed significantly different (*) of 6 morphometric characters at ventral plate by using Independent T-test ($P<0.05$, df1=87, df2=218, df3=305), those were 4 significant characters as L-Ven, L-Anal, W-Anal and L-Epig

Characters	*Tropilaelaps* size average (Mean±S.D.) mm.		
	Tropilaelaps infesting *A. dorsata* (n=88, df1=87)	*Tropilaelaps* infesting *A. mellifera* (n=219, df2=218)	Overall (n=307, df3=305)
Length of ventral plate (L-Ven)*	1.293±0.081	1.252±0.043	1.263±0.060
Width of ventral plate (W-Ven)	0.706±0.053	0.706±0.027	0.706±0.036
Length of anal plate (L-Anal)*	0.289±0.018	0.279±0.018	0.282±0.018
Width of anal plate (W-Anal)*	0.183±0.016	0.190±0.012	0.188±0.014
Length of epigynial plate (L-Epig)*	0.750±0.104	0.788±0.066	0.777±0.081
Width of epigynial plate (W-Epig)	0.144±0.072	0.133±0.011	0.136±0.040

Table 2. Discriminant analysis of Thai tropilaelaps mites performed with significant characters (Variables) and compared between traditional and geometric methods. Results were represented statistical discriminant value from each morphometric methods of the tropilaelaps mite with canonical correlation, Wilks' Lambda and predicted group of infesting their hosts using cross validation

Morphometric Methods	Variables	Standardized canonical discriminant function coefficients	Canonical correlation	Eigenvalue	Wilks' Lambda	Cross validation Predicted group of mites	
						Infesting *A.dorsata*	Infesting *A.mellifera*
Traditioal morphometric	L-Ven	0.559	0.571	0.484	0.674	76.7%	23.3%
	L-Anal	0.426				15.9%	84.1%
	W-Anal	-0.415				Cross-validated group	
	L-Epig	-0.714				78.8%	
Geometric morphometric	warp 1y	-0.398	0.769	1.448	0.409	86.7%	13.3%
	warp7y	0.987				10.9%	89.1%
	warp8x	-0.325				Cross-validated group	
	warp11y\	0.327				88.2%	
	warp21x	0.356					
	warp21y	0.607					
	unix	0.437					

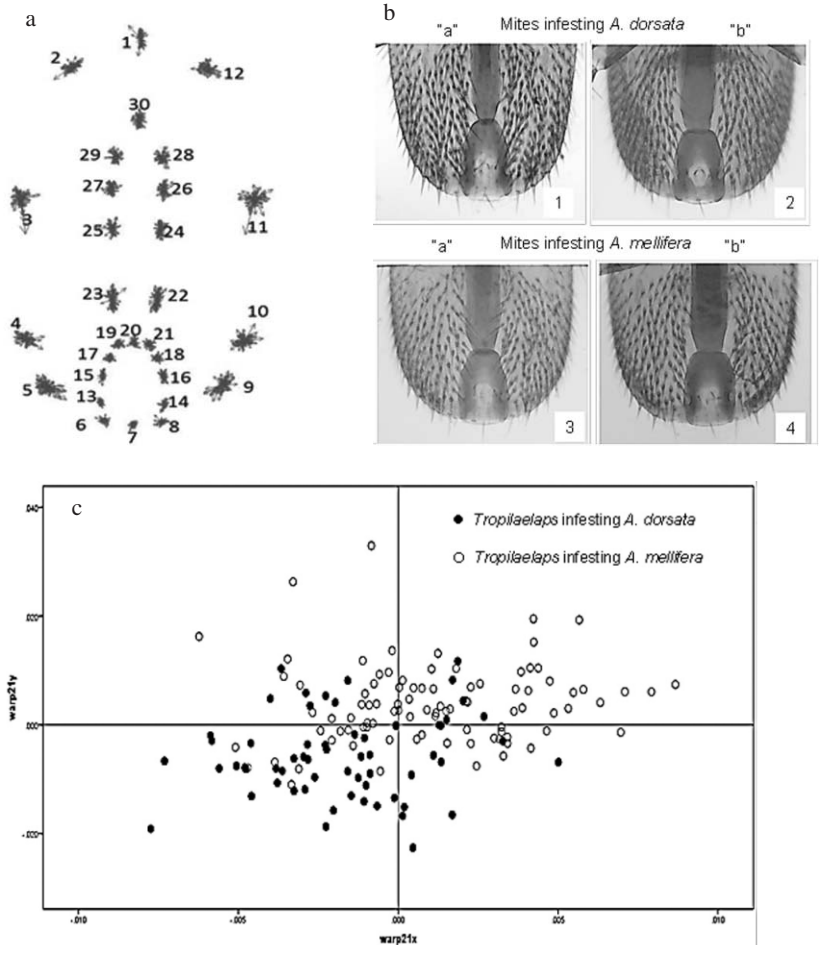

Fig. 2. The *Tropilaelaps* consensus configuration showed vector variation in 30 landmarks (a). The anal plate shape of Thai tropilaelaps infesting *A. dorsata* (1, 2) and *A. mellifera* (3, 4) were as same as *T. mercedesae* following Anderson and Morgan (2007). There were 2 types of anal plate shape, represented with "a" and "b" (b). The PCA of Thai tropilaelaps samples distributed from coordinate axis of X and Y (c). The scatter plots were shown using relative warp analysis of the mite infesting *A. dorsata* (●) and *A. mellifera* (○).

In addition to results of geometric morphometric represented superimposition of 30 landmarks belonging to 152 female tropilaelaps mites, showed the deviation in shape of the mites infesting *A. dorsata* and *A. mellifera* (Fig. 2a). The landmark digitization of Thai tropilaelaps mites were shown consensus configuration presented by the point of the origin vectors. The variation of mites shape was shown highly variances (S^2=0.00006 to 0.00012) at ventral plate shape at the landmark (LM) 1 to 12. While, the potentially LM was shown at anal plate, were highly relative contribution of each landmark (the lowest at LM 6, SS=0.02253 to the highest at LM 20, SS=0.21008). Focus on the analysis demonstrated to describe the morphological variation between Thai tropilaelaps mites infesting different hosts. Furthermore, the relative warp results showed centroid size of Thai tropilaelaps mites, were performed 56 relative warp deformations for the populations. In addition to mahalanobis distance indicated 7 relative warps indicated variability shape of the Thai tropilaelaps mite populations from their infesting different honey bee hosts. Another results of PCA plotting graph showed distribution from coordination of Thai tropilaelaps mite populations of PC1 on X and PC2 on Y axes (Fig. 2c).

All of morphological analysis indicated that Thai tropilaelaps mites were as same as species of *T. mercedaecae*. They showed variation of the mites infesting different hosts in their morphological size and shape. Moreover, the discriminant analysis showed values of the canonical discriminant function were eigenvalue for 1.448, 100% of cumulative and canonical correlation for 0.769. Also, test of function supported with Wilks' Lambda for 0.409. And, cross validation in the geometric shape were correctly classified for 88.2% (Table 2) that higher than the morphometric analysis. However, Thai tropilaelaps mites were similar to *T. mecedecae*, showed variation in their anal shield in both methods.

COI sequences variation of Thai tropilaelaps mites

From morphological data of Thai tropilaelaps mites were identified into *T. mercedesae* ("Tm") based on morphological of anal shield shape following Anderson and Morgan (2007). The COI gene of Thai tropilaelaps mites showed PCR products size approximately 535-538 bp. as same as previously study of genus *Tropilaelaps*. Likewise, restriction pattern of *Dra* I and *Xba* I only digested the PCR products of *T. mecedesae* but was not digest the *T. koenigerum* (Fig. 3). However, RFLP patterns of Thai tropilaelaps mites, *T. mercedesae*, were not different between mites infesting different hosts or the variation of anal shield morphotypes. However, there were 3 samples of Thai tropilaelaps mites infesting *A. dorsata*, identified into *T. koenigerum* ("Tk"). The COI gene variation represented for 5 difference haplotypes of COI sequences within Thai tropilaelaps mites. The comparison of COI sequences of Thai tropilaelaps mites consisted of 3 sequences of *T. koenigerum* infesting *A. dorsata* represented "AdTk" haplotype. The 23 sequences of *T. mercedesae* infesting *A. dorsata* showed 2 difference haplotypes; "AdTma" and "AdTmb" and the 30 sequences of mites infesting *A. mellifera* represented "AmTma" and "AmTmb" haplotypes. The multiple indicated that 4 nucleotides sequences of AdTma, AdTmb, AmTma and AmTmb were closely resemble to the references sequences (EF025445, "TmThailand1EF025445"). Especially, the nucleotide sequences of AmTma and AmTmb showed 100% similarity with the references sequence. But the sequences showed transition substitution at 513 site of T. Otherwise, the sequences of *T. mercedecae* infesting *A. dorsata*, AdTma, showed transition substitution at 200 sites of T and AdTmb showed at 457 sites. Both of them were resemble to *T. koenigerum* sequences. Moreover, the sequences of AdTmb showed transversional substitution at 467 sites of C that was closely related with *T. clareae* (TcMindanao1EF025464) and *T. thaii* (TtVeitnam1EF

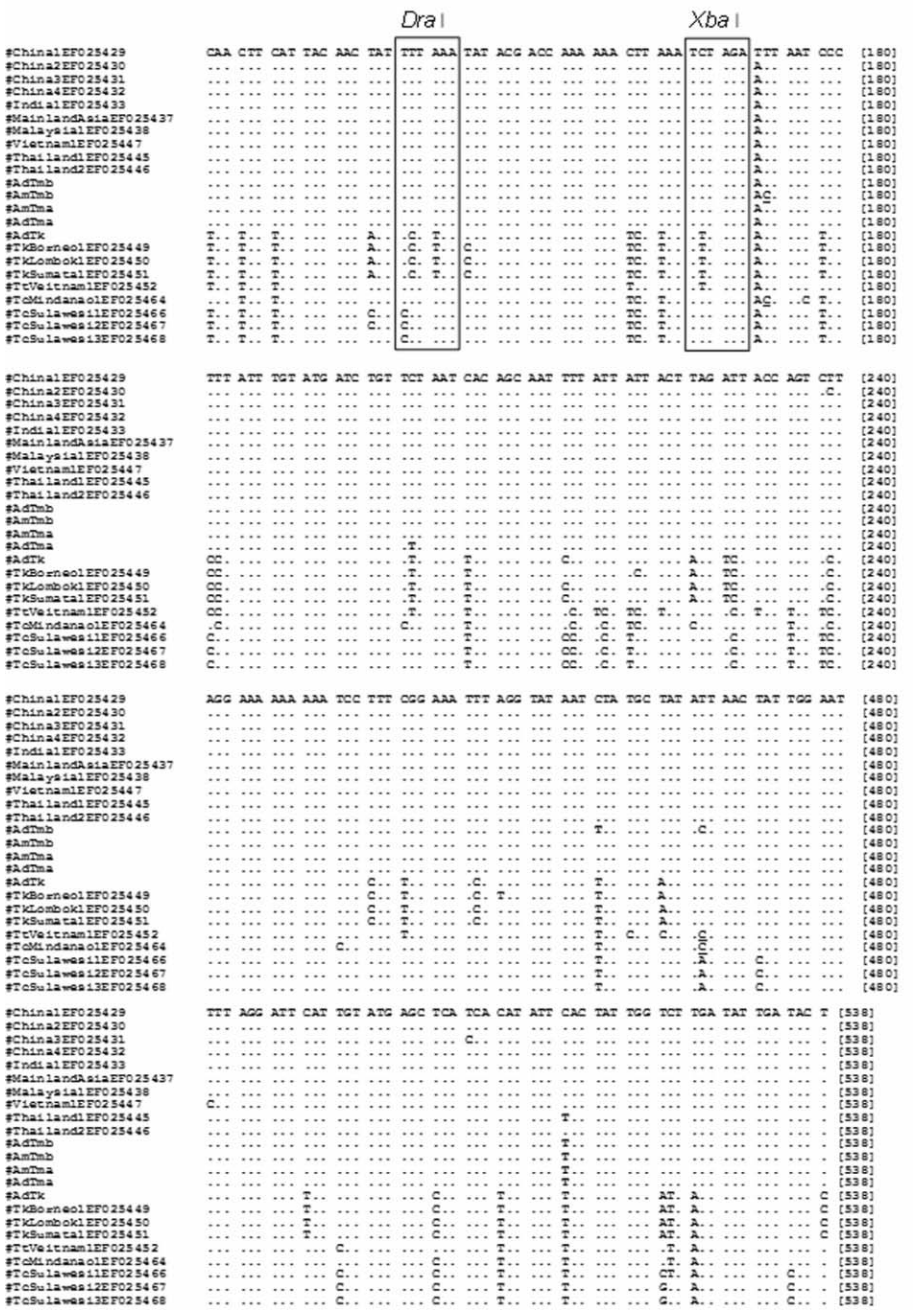

Fig. 3. Multiple alignments of COI gene sequences were compared by 18 *Tropilaelaps* sequences from GenBank database and 5 sequences of Thai tropilaelaps mites (AdTk, AdTma, AdTmb, AmTma and AmTmb) using Mega version 6.0. The partial sequences were shown for their differences. The restriction site of *Dra* I and *Xba* I presented in the box. Also, variable sequences of Thai tropilaelaps mites represented substitution base such as transition showed with bold capital letter and transversion showed with underline bold capital letter.

025452) (Fig. 3 and 4). However, the sequences of AdTk showed 100% similarity with *T. koenigerum.*

Phylogenetic relationship of COI sequences represented similar results which performed under all three criteria as neighbor-joining (NJ) and maximum parsimony (MP), additional statistical tested with bootstrap 1,000 replications. Almost all Thai tropilaelaps mites infesting *A. dorsata* and *A. mellifera* were same as *T. mecedesae* sequences from database and found a small proportion *T. koenigerum* sequences. But the investigation was not found

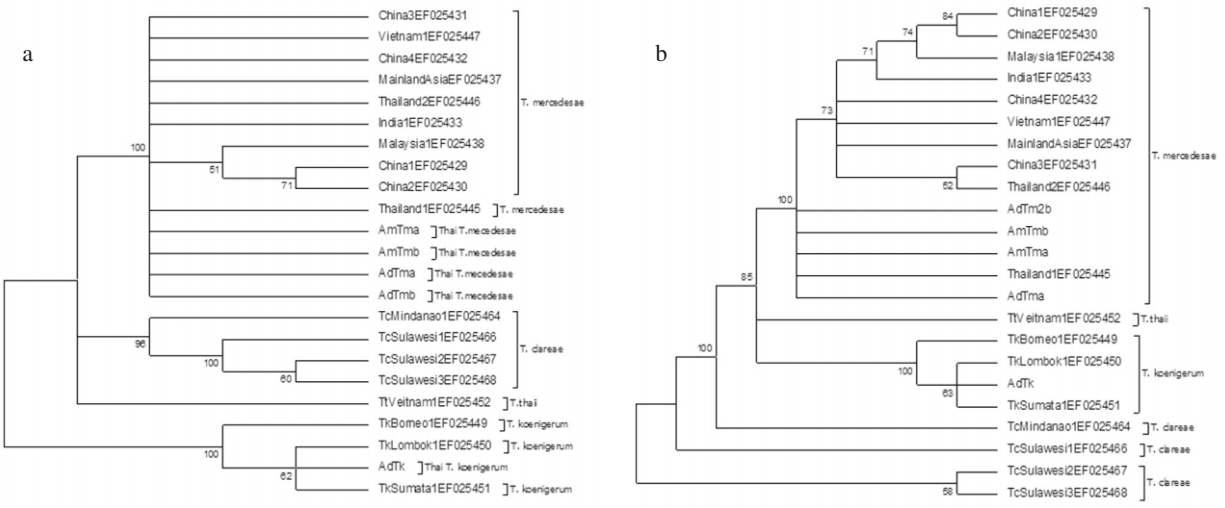

Fig. 4. Phylogenetic tree reconstructed based on 538 bp. COI sequences of five Thai tropilaelaps and GenBank sequences. NJ and MP methods were performed with bootstrap 1,000 replications and calculated 50% majority cut-off tree branching by using Mega 6.0 program. The tree results of NJ (a) and MP (b) indicated that 4 Thai tropilaelaps sequences (AdTma, AdTmb, AmTma and AmTmb) clustered in T. mercedesae clade and one sequences (AdTk) was in *T. koenigerum* clade. In detail of NJ tree (a) reconstructed by p-distance and Kimura-2-Parameter (K2P), they were similarity in their relationship (SBL=0.375. And, MP tree (b) were random addition of 10 of no. of initial trees, CI=0.705, RI=0.883, RCI=0.623.

T. clareae or *T. thaii* sequences. However, the tree indicated that 4 sequences of Thai tropilaelaps mites grouped into the *T. mercedesae* clade were mostly resemble to sequences accession number EF025445. While, the mites infesting *A. dorsata* from Lampang province of Thailand "AdTk" sequences clustered into *T. koenigerum* clade. The phylogenetic of neighbor-joining were performed with p-distance and K2P. Unweighted NJ tree were generated bootstraps for 1,000 replications and 50% majority rule consensus and condense tree showed SBL for 0.342 (Fig. 4a). In the maximum parsimony was shown most parsimonious trees length (207). The consistency index (CI) was 0.670 the retention index (RI) was 0.883 and the composite index (RCI) was 0.623 (Fig. 4b).

DISCUSSION

The major of Thai tropilaelaps mites infesting *A. dosata* were identified into *T. mecedesae*. We did not detect *T. clareae* and *T. thaii*. But, we found 3 mites infesting *A. dorsata* were small in body size and pear shape anal plate similar to the findings of other reports in context to *T. koenigerum* morphology (Anderson and Morgan, 2007; Kavinseksan and Wongsiri, 2016). Mophological variation of the female *Tropilaelaps* was found only *T. mercedesae* infesting different hosts of *A. dorsata* and *A. mellifera*. There was significantly morphological variation in the anal and epigynial plate (Anderson and Morgan, 2007). The measurements of traditional and geometric morphometric on ventral plate image by using tpsDig 2.04 are effective identification Thai tropilaelaps mites. We suggested that the Thai tropilaelaps mites, *T. mercedesae*, infesting different hosts (*A. dorsata* and *A. mellifera*), showed different size and shape of anal and epigynial plate. There were two forms ("a" and "b") at the apex of anal plate in each mite infesting differently hosts. The "a" form showed as bell shape (infesting *A. dorsata*), slightly blunt shape (infesting *A. mellifera*) and "b" showed wide bell shape (infesting *A. dorsata*) and wide blunt shape (infesting *A. mellifera*) (Fig. 2b). Almost of *T. mercedesae* were large size and varies shape from blunt to sharp at the anterior margin of anal and the apex of the epigynial plate. Those showed overlapping of the both plates in adult female mites that found to be artefact caused by mounting on glass

Table 3. The genetic distance estimation between groups was performed using p-distance method in 23 COI sequences using Mega version 6.0. There were 6 groups such as *T. clareae, T. mercedesae, T. koenigerum* and *T. thaii* followed COI sequences database (Anderson and Morgan, 2007; Luo *et al*., 2011). And, there were Thai *T. mercedaecae* sequences (AdTma, AdTmb, AmTma and AmTmb) and Thai *T. koenigerum* (AdTk). Overall mean distance estimation for substitution (d) was 2.147. The percentages of distance estimation overall (%) and between groups represented lower than 0.05 in the value (bold number*) supported in their closely related of the COI sequences in each *Tropilaelaps* groups. The pairwise distance of sequences divergence showed overall estimate distance for 8.3%.

Mites	*T. mercedecae*	Thai *T. mercedecae*	Thai *T. koenigerum*	*T. koenigerum*	*T. clareae*	*T. thaii*
T. mercedecae	0.000					
Thai *T. mercedecae*	0.007* (0.7)	0.000				
Thai *T. koenigerum*	0.146 (14.6)	0.142 (14.2)	0.000			
T. koenigerum	0.147 (14.7)	0.143 (14.3)	0.008* (0.8)	0.000		
T. clareae	0.125 (12.5)	0.124 (12.4)	0.141 (14.1)	0.144 (14.4)	0.000	
T. thaii	0.119 (11.9)	0.114 (11.4)	0.133 (13.3)	0.136 (13.6)	0.110(11.0)	0.000

slide.

Recently, the geometric morphometric were clearly indicated variation, relationship and their biogeography of the mite populations such as coconut mites, *Aceria guerreronis* (Navia *et al*., 2005; 2006) and oppiid mites from Turkey (Baran *et al*., 2011). The geometric method is powerful to determining shape variation of *T. mercedesaae* infesting different hosts that was firstly utilized in the female Thai *tropilaelaps* mites. The result suggested that 30 landmarks on ventral plate showed relative deformation of body shape, anal and epigynial plate variation. Likewise the deformation of coxigenital and ventral regions variation of *Aceria guerreronis* populations suggests morphometric geometric method using for mite identification (Navia *et al*., 2006). Furthermore, traditional and geometric methods separated the oppiid mites in genera taxa quite well. But the results of geometric morphometric considered these mites as subgenera such as the genus *Oppiella* (Baran *et al*., 2011). These results considered *Tropilaelaps* mites populations infesting different hosts, *A. dorsata* and *A. mellifera*, they were overlapping populations of *T. mercedesae* (Fig. 2c). However, discriminant analysis indicated that they may be grouped their morphological related to their hosts. And, geometric method was higher percentage of cross-validation to classification than traditional method (Table 2). Although, the analyses conducted in this investigation suggest that geometric methods may be useful in understanding morphological variability of *Tropilaelaps* mites.

The COI sequences of Thai tropilaelaps mites were reported for 5 haplotypes, especially one of *T. koenigerum* infesting *A. dorsata* from Thailand which differed from previous investigation (Anderson and Morgan, 2007; Luo *et al*., 2011.). In Thailand, *T. koenigerum* was reported RFLP markers of ITS gene in mitochondrial DNA to identify mites. The ITS polymorphism was not appropriate for genetic diversity of *Tropilaelaps* spp. at the intraspecific level (Tangjingjai *et al*., 2003). But the result of COI sequences variation of Thai tropilaelaps mites indicated clearly of two distinct species of mites infesting *A. dorsata* such as *T. koenigerum* and *T. mercedesae*. While, the Thai tropilaelaps mites infesting *A. mellifera* indicated *T. mecedesae*. The pairwise distance of COI sequences were estimated evolutionary divergence (Tamura *et al*., 2011) within and between groups. These results of demonstrated percentage of genetic distance within and between groups were range from 1.2 to 4% and 0.11.1 to 14.7%. Similar to Anderson and Morgan (2007) reports pairwise distances of *Tropilaelaps* was within 1.2~3.5% and between are 11.1 to14.7%. Moreover, genetic distance of COI gene in the other Acari have shown 2.5% and 16% sequences divergence within and between species of genus *Stratiolaelaps* as same family as *Tropilaelaps*. And percentages of sequences divergence were 0~2.1% and 6.1~9.1% within and between species of *Varroa* as same order as *Tropilaelaps* (Anderson and

Trueman, 2000). We concluded that the divergence distance within and between COI sequences haplotypes of these Thai tropilaelaps mites indicated 2 species such as Thai *T. mercedesae* and Thai *T. koenigerum*. Almost of *Tropilaelaps* mites considered species of *T. mercedesae* which have common found infesting naturally *A. dorsata* and domestically *A. mellifera* colonies of Thailand. Moreover, phylogenetic relationship of NJ and MP tree with bootstrap analysis supports this conclusion.

Recently, the beekeeping of *A. mellifera* in Thailand has found *Tropilaelaps* infections as same as other countries which wondered the problem of colony collapse disorder (CCD) such as in North America (vanEngelsdorp *et al*., 2009). But, there were no reports of CCD in Thailand. We are wondering about the CCD problem which has a little understanding of the causes. Parasitic bee mites, *Varroa* and *Tropilaelaps*, infection can lead to weakening of the colony. Such colonies have diminishing amounts of brood and adult bees causing collapses of the colonies and *T. mercedesae* can kill untreated colonies of *A. mellifera* (Kavinseksan and Wongsiri, 2016).

In conclusion, these results may be shown detail of methods to identify Thai tropilaelaps species using both of morphological and molecular analysis. Which have shown two mites species (*T. koenigerum* and *T. mercedesae*) infesting on native host (*A. dorsata*) and introduced host (*A. mellifera*). A little number of *T. koenigerum*, infected only *A. dorsata*, which do not find these mites in *A. mellifera* colonies. These species suggests clearly host-parasite relationship. While, *T. mercedesae* can be found widely infesting on honey bees host. They can great colonize *A. mellifera* colonies which may be destroyed honey bee development, adult disorder or cause of pathogen transmission. These results suggest that *T. mercedesae* has been variability in their morphology such as anal plate and COI sequences. The relationship of *T. mercedesae* infesting difference bee hosts has shown high variation in both data. Reasonable, the *T. mercedesae* shifted host to *A. mellifera*, they may be well adaptation and living with the newly environment. So *T. mercedesae* has become major pest in *A. mellifera* beekeeping in Thailand and the world. However, the successful morphological marker (traditional and geometric methods) and molecular markers (COI sequences) demonstrates the powerful techniques to identify Thai tropilaelaps mites. These markers are useful for tracing the colonization of newly bee hosts and are benefit for beekeeping of Thailand or other countries.

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