ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol Vol. 53, No. 3: 315-320, June 2015
http://dx.doi.org/10.3347/kjp.2015.53.3.315

▣ ORIGINAL ARTICLE

Molecular Prevalence of *Acarapis* Mite Infestations in Honey Bees in Korea

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Abstract: *Acarapis* mites, including *Acarapis woodi*, *Acarapis externus*, and *Acarapis dorsalis,* are parasites of bees which can cause severe damage to the bee industry by destroying colonies and decreasing honey production. All 3 species are prevalent throughout many countries including UK, USA, Iran, Turkey, China, and Japan. Based on previous reports of *Acarapis* mites occurring in northeast Asia, including China and Japan, we investigated a survey of *Acarapis* mite infestations in honey bees in Korean apiaries. A total of 99 colonies of *Apis mellifera* were sampled from 5 provinces. The head and thorax of 20 bees from each colony were removed for DNA extraction. PCR assays were performed with 3 primer sets, including T, A, and K primers. Results indicated that 42.4% (42/99) of samples were *Acarapis*-positive by PCR assay which were sequenced to identify species. Each sequence showed 92.6-99.3% homology with reference sequences. Based on the homology, the number of colonies infected with *A. dorsalis* was 32 which showed the highest infection rate among the 3 species, while the number of colonies infected with *A. externus* and *A. woodi* was 9 and 1, respectively. However, none of the *Acarapis* mites were morphologically detected. This result could be explained that all apiaries in the survey used acaricides against bee mites such as *Varroa destructor* and *Tropilaelaps clareae* which also affect against *Acarapis* mites. Based on this study, it is highly probable that *Acarapis* mites as well as *Varroa* and *Tropilaelaps* could be prevalent in Korean apiaries.

Key words: *Acarapis* mite, molecular prevalence, *Apis mellifera* (bee)

INTRODUCTION

Acarapis mites are parasites of bees which can cause severe damage to the bee industry by decreasing honey production, destroying colonies in both managed and feral honey bees and decreasing pollination [1]. Currently there are 3 species of *Acarapis* mites: *A. woodi*, *A. externus*, and *A. dorsalis* [2]. These mites are known to parasitize specific locations on bees. *A. externus* is mainly found on the external surface of the head and thorax of bees. *A. dorsalis*, on the other hand, is mainly found on the thorax especially in a groove between the mesoscutum and mesoscutellum of bees. Unlike these 2 species, *A. woodi* is an internal parasite that lives in the abdominal and thoracic air sacs of bees [3]. All stages of the mite live within the tracheae, except during a brief period when adult females disperse to search for new hosts [3].

Each *Acarapis* mite shows different pathogenicity according to its habitat. Although *A. externus* has been reported to cause wing loss or malfunction, the 2 species of external mites, *A. externus* and *A. dorsalis*, generally do little damages [1]. Unlike the other 2 ectoparasitic mites, *A. woodi* causes blockage of thoracic tracheae, reducing the diffusion of oxygen to the flight muscles and to the brain, and serve as a vector of other pathogens such as bacteria and viruses [3]. Moreover, heavy infestation of the mite causes a shortened lifespan of bees, a diminished brood [4], smaller bee populations, looser winter clusters, increased honey consumption, lower honey yields, and ultimate colony demise [3].

All 3 species of the genus *Acarapis* are distributed throughout many countries. *A. dorsalis* has been reported on *Apis mellifera* from Europe, Canada, USA, New Zealand, Australia, Papua New Guinea, and Hawaii. The world distribution of *A. externus* is the similar to that of *A. dorsalis*, but *A. externus* is collected more frequently and more abundant than *A. dorsalis* [2]. *A. woodi* has been reported in many European countries, in-

• Received 4 January 2015, revised 6 May 2015, accepted 30 May 2015.
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* Sequence data from this article have been deposited with the DDBJ Data Libraries under accession nos. LC 006083-5.

cluding Scotland, France, Spain, and Greece, after its first report in 1919. The mite then spread to the American continent, first to Argentina, Colombia, Mexico, and USA. Outside the European and American continents, it was reported in Iran, Turkey, China, and Japan [2,5-7]. Because Korea is located between China and Japan, *Acarapis* mites are expected to be present in Korea. However, the presence of *A. woodi* in Korea has not yet been reported.

In Korea, many studies on parasitic bee mites have been reported, including the infestation status and control methods on *Varroa jacobsoni* and *Tropilaelaps clareae* [8]. However, there have not yet been studies on their occurrence. If an outbreak of the mites suddenly happened, this present situation could cause bee colony devastation and *Acarapis* mite-mediated diseases on the Korean apicultural industry. We used a PCR detection method specific to *Acarapis* mites and surveyed the prevalence of *Acarapis* mites.

MATERIALS AND METHODS

Sample collection

A total of 99 *Apis mellifera* colonies were sampled from 72 apiaries in 5 provinces (Gyeonggi, Gangwon, Chungcheong, Gyeongsang, and Jeolla) in Korea (Fig. 1). Fifty to 100 adult bees were collected and pooled from 1-2 colonies in each apiary between August and December 2013. The honey bee samples were collected from brood nests inside the hives for managed colonies and at the hive entrances for feral colonies, respectively [5]. After collecting the bees, the samples were stored at -20˚C for PCR detection.

PCR detection and sequencing

The head and thorax of 20 adult bees randomly chosen from each hive were used for DNA extraction using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Total DNA was eluted in 30 µl of elution buffer and used for PCR with LeGene Hot Start PCR Mix w/Sky Blue Dye (LeGene Biosciences, San Diego, California, USA). Two primer sets, including *Acarapis* (A) primer and Kojima (K) primer set, were used to identify *Acarapis* mite (Table 1) which were capable of amplifying mitochondrial cytochrome *c* oxidase subunit I (*COI*) DNA fragments of *Acarapis* mites [5,9,10]. These primers do not amplify honey bee *COI*

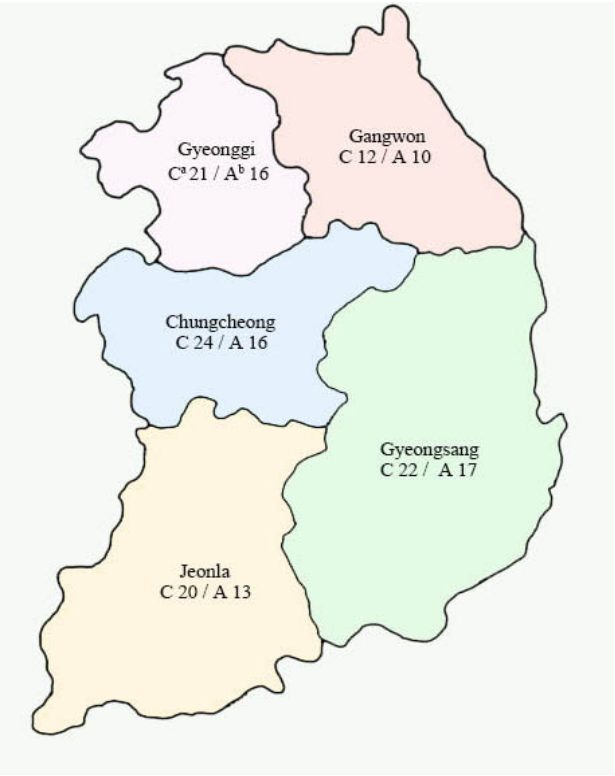

Fig. 1. Distribution of apiaries visited during the survey. [a]C indicates the number of collected colonies and [b]A is the number of visited apiaries in each province.

Table 1. Primer sets for *Acarapis* mite detection

Name for primer sets	Sequence (5' to 3')[a]	Length (bp)	References
Kojima (K)[b]	F- CAGTAGGGCTAGATATCGATACCCGAGCTT R- TGAGCTACAACATAATATCTGTCATGAAGA	247	Kojima et al. [5]
Acarapis (A)[c]	F- CGGGCCCGAGCTTATTTTACTGCTG R- GCGCCTGTCAATCCACCTACAGAAA	162	Garrido-Bailón et al. [9]
AmHsTRPA (T)[d]	F– CACGACATTCAAGGTTTAAGAAATCACG R- TCAGTTATTCTTTTCCTTTGCCAGATTT	426	Kojima et al. [5]

[a]F: forward primer; R: reverse primer.
[b]Kojima (K); the primer set which amplifies mitochondrial cytochrome *c* oxidase subunit I (*COI*) DNA fragments of *Acarapis* mites.
[c]*Acarapis* (A); the primer set which amplifies *COI* DNA fragments of *Acarapis* mites.
[d]AmHsTRPA (T); the primer set which amplifies a honey bee genomic DNA fragment encoding a part of AmHsTRPA.

DNA fragments; thus, total DNA isolated from bees was used as a template for PCR. As a positive control for PCR to verify the quality of DNA extraction, a honey bee genomic DNA fragment encoding a part of a honey bee Hymenoptera-specific transient receptor potential A channel (AmHsTRPA) was amplified by AmHsTRPA (T) primer set [10]. The thermal cycling conditions were as follows: 1 cycle of initial denaturation at 94˚C for 2 min, 35 cycles of denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec, and extension at 72˚C for 30 sec. The PCR product was analyzed by 2% agarose gel electrophoresis. A negative control lacking template DNA was performed for each PCR reaction. Since A and K primer sets equally amplify *COI* genes of 3 species of *Acarapis* mites (*A. woodi*, *A. dorsalis*, and *A. externus*), the amplified products with T and A primer sets or T and K primer sets were sequenced and aligned with the sequences deposited in GenBank to determine the parasitized mite species. The nucleotide sequences were identified by the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI). Multiple nucleotide alignment was carried out using the published SBV sequences as references by BioEdit version 7.0.9.0 (Table 2). The phylogenetic tree was constructed by Mega 6.06.1 software [11] using the neighbor-joining (NJ) method [6].

Microscopic examination of *Acarapis* mite

To confirm the presence of *Acarapis* mites, a total of 20 bees per each colony were examined with a modified dissection method developed by Lorenzen and Gary [12]. The external surface, including the head, thorax and a groove between the mesoscutum and mesoscutellum of each bee, was examined to find out *A. externus* and *A. dorsalis*. To examine *A. woodi*, each bee was pinned through the thorax into bee wax culture dish and placed under a dissecting microscope. The head and the first pair of legs were removed off using a scalpel or razor blade. The ring of prothoracic sclerite (collar) was also removed using fine forceps to fully expose both left and right sides of the trachea. The exposed trachea of 2 right and left sides was examined with 20× magnification to find out tracheal color change, the body of *A. woodi* and foreign substance. If the presence of *A. woodi* or foreign substance was confirmed, the trachea was detached and placed on a glass slide and examined under an optical microscope to diagnosis *A. woodi*.

RESULTS

Prevalence of *Acarapis* mite infestations

A total of 99 colonies from 72 apiaries in 5 provinces (Gyeonggi, Gangwon, Chungcheong, Gyeongsang, and Jeolla) were examined (Fig. 1). All of the 99 colonies were T primer set positive. Among these, 36, 2, and 4 colonies were positive for A, K, and both A and K primer sets, respectively (Table 2). The infection rate of *Acarapis* mites in Gangwon, Gyeonggi, Chungcheong, Gyeongsang, and Jeolla were 25.0%, 9.5%, 25.0%, 95.5%, and 50.0%, respectively (Table 2). Gyeongsang had the highest infection rate, followed by Jeolla, Chungcheong, and Gyeonggi. Gangwon had the lowest infection rate among the 5 provinces. Between the primer sets, the A primer set was more sensitive than the K primer set (Table 2).

Molecular characterization of Korean *Acarapis* mites

To confirm the above PCR assay results, all 42 *Acarapis*-positive colonies were sequenced and aligned. Each sequence showed 92.6-99.3% homology with reference sequences available in GenBank (Table 3). Species identification of each colony was determined if the sequence between the unknown sample and the reference showed 98-100% homology. Based on these criteria, the number of colonies infected with *A. dorsalis* was 32 samples which showed the highest infection rate

Table 2. PCR assay result for the bee colonies

Province	No. of tested colonies	Number of positive colonies (%)[a]			
		A primer set	K primer set	A and K primer sets	Total
Gangwon	12	3 (25.0)	0 (0)	0 (0)	3 (25.0)
Gyeonggi	21	1 (4.8)	1 (4.8)	0 (0)	2 (9.5)
Chungcheong	24	5 (20.8)	0 (0)	1 (4.2)	6 (25.0)
Gyeongsang	22	19 (86.4)	1 (4.5)	1 (4.5)	21 (95.5)
Jeonla	20	8 (40.0)	0 (0)	2 (10.0)	10 (50.0)
Total	99	36 (36.4)	2 (2.0)	4 (4.0)	42 (42.4)

[a](%) = (no. of positive colonies / no. of tested colonies with PCR assay) × 100.

Table 3. Sequence relationships of the nucleotide of mitochondrial cytochrome c oxidase subunit I (*COI*) DNA fragments from Korean isolates of Acarapis mites

Species	Country	Accession#	Nucleotide sequence identity (%)												
			A. dorsalis New Zealand	*A. dorsalis* Canada	*A. dorsalis* New Zealand	*A. externus* New Zealand	*A. externus* New Zealand	*A.externus* United Kingdom	*A. woodi* Canada	*A. woodi* United Kingdom	*A. woodi* USA	*A. woodi* Korea	*A. dorsalis* Korea	*A. externus* Korea
		HQ243439	GQ916567	HQ243434	HQ243441	HQ243440	FJ603293	GQ916565	FJ603296	EU190886	LC006084	LC006085	LC006083
A. dorsalis	New Zealand	HQ243439	100										
A. dorsalis	Canada	GQ916567	99.3	100									
A. dorsalis	New Zealand	HQ243434	100	99.3	100								
A. externus	New Zealand	HQ243441	93.2	93.8	93.2	100							
A. externus	New Zealand	HQ243440	93.2	93.8	93.2	100	100						
A. externus	United Kingdom	FJ603293	93.2	93.8	93.2	100	100	100					
A. woodi	Canada	GQ916565	96.3	95.7	96.3	94.4	94.4	94.4	100				
A. woodi	United Kingdom	FJ603296	96.3	95.7	96.3	94.4	94.4	94.4	100	100			
A. woodi	USA	EU190886	96.3	95.7	96.3	94.4	94.4	94.4	100	100	100		
A. woodi	Korea	LC006084	96.9	96.3	96.9	93.8	93.8	93.8	99.3	99.3	99.3	100	
A. dorsalis	Korea	LC006085	99.3	98.7	99.3	92.6	92.6	92.6	96.9	96.9	96.9	97.5	100
A. externus	Korea	LC006083	92.6	93.2	92.6	99.3	99.3	99.3	95	95	95	94.4	93.2	100

among the 3 species, while the number of colonies infected with *A. externus* and *A. woodi* was 9 and 1, respectively. The DNA sequences of PCR-amplified *CO*1 fragments infested by *A. dorsalis A. externus*, and *A. woodi* were deposited to a DDBJ database as the no. LC006085, LC006083, and LC006084, respectively. Although *A. externus* and *A. dorsalis* were distributed throughout all 5 provinces, *A. woodi* was only detected from an apiary in Mungyung City, Gyeongsang (Gyeongsangbuk-do Province) (Table 4). The phylogenetic analysis between Korean isolates in this study and other references was performed (Fig. 2). Each Korean isolate *Acarapis* species was placed on a separate branch and closely related to the same *Acarapis* species.

Morphological diagnosis of *Acarapis* mites

In microscopic examinations, a total of 20 bees per each colony were dissected, but no diagnosable mite body was detected. However, foreign bodies which assumed the mite and some debris were discovered from the *A. woodi*-positive bee colony (Fig. 3).

DISCUSSION

In this study, the prevalence of *Acarapis* mites was surveyed by PCR and subsequent sequencing. Molecular analysis of bee DNA extracts using PCR and alignment indicate that all 3 species of *Acarapis* mites are present in Korea and 32.3% (32/99) colonies were infected with *A. dorsalis*, 9.1% (9/99) with *A. externus*, and 1.0% (1/99) with *A. woodi* from 99 colonies in Korea. Although we molecularly identified *Acarapis* mites, the *Acarapis* mites were not found in microscopic examinations. This result could be explained by the fact that all apiaries in the survey used in-house type acaricides against bee mites such as *Varroa destructor* and *Tropilaelaps clareae* which also affected against *Acarapis* spp. Reports also indicate that most apiaries of Korea regularly use insecticides for acarine control [13,14]. Therefore, only dead bodies of the mites or debris might have remained and been detected by PCR assay (Fig. 3).

While *A. dorsalis* and *A. externus* were detected in all 5 provinces, *A. woodi* was found only in 1 apiary farm, and the infection rate was very low. Species-specific seasonal fluctuation could explain this phenomenon. In *A. dorsalis* and *A. externus*, the incidence of these mites decreased in fall, increases in spring, and is highest during May and September in temperate regions [15,16]. On the other hand, the prevalence of *A. woodi* increased greatly from November to February and declined in

Table 4. Regional occurrence of *Acarapis* spp. in *Apis mellifera* from Korea

Province	No. of tested colonies	No. of positive colonies (%[a])			
		A. dorsalis	*A. externus*	*A. woodi*	Total
Gangwon	12	2 (16.7)	1 (8.4)	0 (0)	3 (25.0)
Gyeonggi	21	2 (9.5)	0 (0)	0 (0)	2 (9.5)
Chungcheong	24	6 (25.0)	0 (0)	0 (0)	6 (25.0)
Gyeongsang	22	14 (63.6)	6 (27.3)	1 (4.5)	21 (86.3)
Jeonla	20	8 (40.0)	2 (10.0)	0 (0)	10 (50.0)
Total	99	32 (32.3)	9 (9.1)	1 (1.0)	42 (42.4)

[a](%) = (no. of positive colonies / no. of tested colonies with PCR assay) × 100.

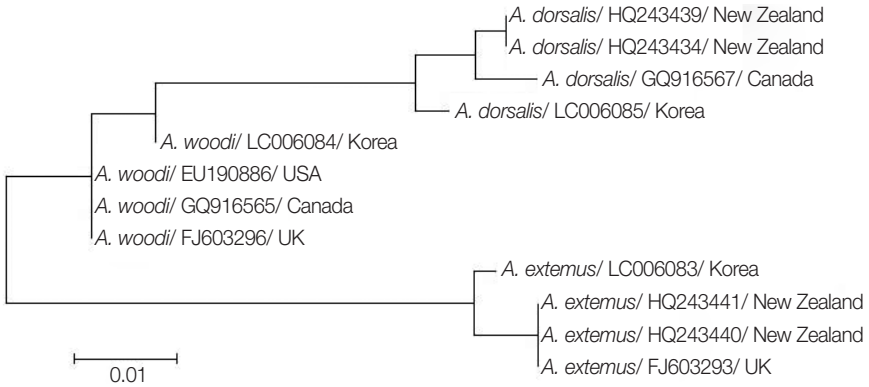

Fig. 2. Phylogenetic tree of the nucleotide of mitochondrial cytochrome *c* oxidase subunit I (*COI*) DNA fragments from Korean isolates of *Acarapis* mites and other refer ences of *Acarapis* mites.

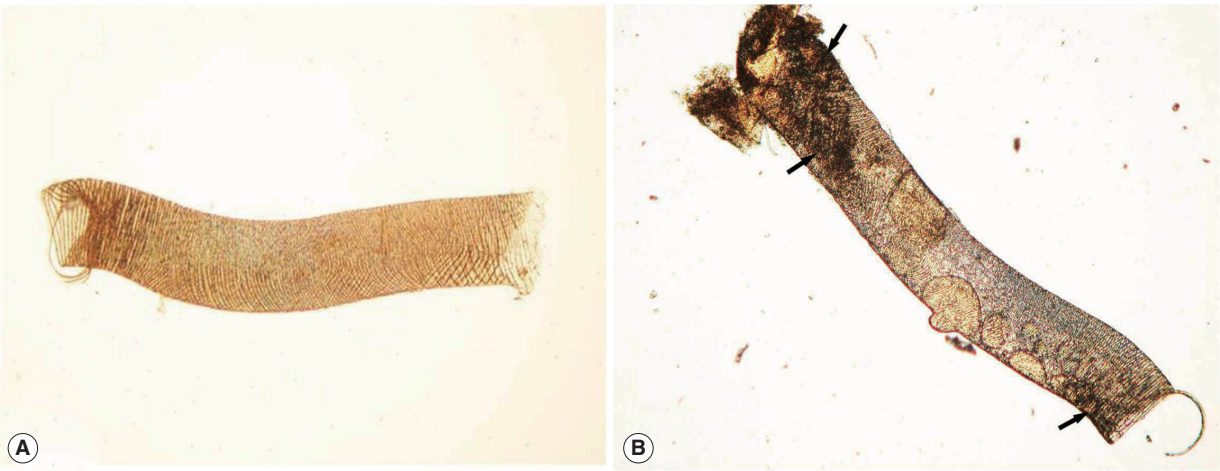

Fig. 3. Light microscopic view of a bee trachea. (A) Clean trachea from healthy bee. (B) Trachea from *Acarapis woodi* positive bee by sequence analysis. Foreign bodies which assumed the mite and some debris were discovered (arrow).

late spring [17]. We collected most of the bee samples during August and October. Consequently, the population of *A. woodi* might be too low for detection. In future studies, the bee samples should be collected during fall and winter seasons and examined to confirm the seasonal fluctuation of *Acarapis* mites in Korea.

In general, the most common diagnostic method for *Acarapis* mite infestation is morphological identification. Further, the molecular prevalence of *Acarapis* mites has been conducted only in a few countries [14]. However, the morphological

identification is generally time-consuming and requires detailed and sustained attention by the screener. On the other hand, molecular methods facilitate rapid and sensitive detection of *Acarapis* spp. in many honey bee samples for epidemiologic surveys [10]. Therefore, we adopted 2 different genus-specific primers, including K and A primer sets. Our results indicated that the A primer set was more sensitive than the K primer set. However, both A and K primer sets require sequencing of amplicons to identify the species of the mite. Therefore, there is a need to design a primer which can amplify species-specific *COI* DNA fragments in future studies.

ACKNOWLEDGMENTS

This study was supported by the Animal and Plant Quarantine Agency (grant no. Z-1543081-2013-13-01), Korean Ministry of Agriculture and Forestry, Republic of Korea.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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