Original Article

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SEM Observations of Korean Haloptype *Varroa destructor* (Acari: Varroidae) Collected from *Apis mellifera* Colonies

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Abstract

Scanning electron microscopic observations on the Korean haplotype of *Varroa destructor* collected from *Apis mellifera* was indentifed by mtDNA analysis to describe external morphology K-type *V. destructor*. SEM micrographs showed that the K-type *V. destructor* is more oval than Japan/Thai-Vietnam type and has three kinds of setae *viz*, serrated, spiny, and smooth marginal setae. The marginal setae are somewhat flexible. The K-type *V. destructor* is larger than *V. jacobsoni* and *V. rindereri*. The peritreme of K-type *V. destructor* is smaller than *V. jacobsoni* and *V. rindereri*. No sternal pores were observed in the K-type *V. destructor*, which is present in *V. jacobsoni* and *V. rindereri*. The K-type *V. destructor* has a more metapodal setae than *V. jacobsoni* and *V. rindereri*.

Key words: SEM micrographs, K-type *Varroa destructor*, Korea

INTRODUCTION

The parasitic mite, *Varroa destructor* Anderson and Trueman (2000), is the most important mite in apiculture (De Jong, 1997) due to its destructive nature of honeybees (Rinderer *et al*., 2001). Two types of haplotypes *V. destructor* mites; the Korean haplotype *Varroa destructor* (K-type) from Korea and the Japanese haplotype (J-type) from Japan, cause brood damage to the European honey bee, *Apis mellifera* (Anderson and Trueman, 2000; Garrido *et al*., 2003; Solignac *et al*., 2005). The K-type *Varroa destructor* is more oval than the J-type (de Gunzman *et al*., 1997; Anderson and Trueman, 2000; Garrido *et al*., 2003).

The K-type *V. destructor* have successfully infested *A. mellifera* brood (Anderson and Trueman, 2000). The K-type *V. destructor* also shifted from *A. cerana* to *A. mellifera* near Vladivostok (north Korean Penninsula) from Ukraine during late 1950s (de Guzman *et al*., 1997; Oldroyd, 1999), whereas the J-type *V. destructor* first shifted from *A. cerana* to *A. mellifera* in Japan during the previous century after introduction of *A. mellifera* (Sakai and Okada, 1973) and then spread to *A. mellifera* in Thailand and Brazil (Anderson and Trueman, 2000) and then in North America (de Guzman *et al*., 1997). Varroa mites reproduce in the cells with developing honeybee larvae of eastern honeybee, *Apis cerana* and the European honeybee, *Apis mellifera*. They feed on the haemolymph of developing larvae and adult bees, resulting in the

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transmittion of viruses and bacteria (Rosenkranz *et al*., 2010). Infestation rates of the K-type *V. destructor* are high that were found in all major beekeeping countries except Australia (USDA, 1987; Bradbear, 1988; Matheson, 1995). This ectoparasite feeds on the hemolymph of adult bees and the larvae and is subjected to microbial invasion (De Jong and De Jong, 1983), bacteria and viruses (Koch and Ritter, 1989; Shimanuki *et al*., 1992), especially acute paralysis virus (Ball, 1985; Allen *et al*., 1986), causing reduced life (De Jong and De Jong, 1983) and collapse of the infested colonies within one to three years (Chen and Siede, 2007; Cox-Foster *et al*., 2007; Johnson *et al*., 2009). *V. destructor* is a highly destructive pest which is responsible for reduced honey and brood production (Delfinado-Baker, 1988).

Previous studies on *V. destructor* have concentrated on distribution (Munoz, *et al*., 2008), host-parasite relations (de Gunzman *et al*., 1997; Garrido *et al*., 2003; Piccirillo and De Jong, 2003), genetic variation (Anderson and Fuchs, 1998; de Guzman and Rinderer, 1999; Anderson, 2000; Anderson and Trueman, 2000; Solignac *et al*., 2005), mite infestation (de Guzman *et al*., 1997; Anderson and Fuchs, 1998), and hygienic behavior of bees against *V. destructor* (Harbo and Harris, 2005; Spivak and Reuter, 2001). Previous descriptions of *V. destructor* have been brief and most of the available information on *V. destructor* exists at genetic level (Anderson and Trueman, 2000; Solignac *et al*., 2005).

The body size, body setae, marginal setae, chelicerae, palps, peritreme, and legs have not yet been described for the K-type *V. destructor*. Although mtDNA can be used to identify the K-type *V. destructor* (Solignac *et al*. at 2005), a simple technical method is desirable. Identification of *V. destructor* by using the mtDNA analysis is far beyond the capabilities of extension agents and beekeepers. Our knowledge on *V. destructor* morphology and behavior is still too fragmentary to lead to a satisfying controlling method. Here, we describe the morphology of the K-type *V. destructor* using SEM in combination with mtDNA analysis and compare morphological features of K-type *V. destructor* with published studies to clearly understand the K-type *V. destructor* mites. SEM micrographs of the K-type *V. destructor* will be helpful for accurate identification required for eradication.

MATERIALS AND METHODS

Sampling

Adult K-type *V. destructor* mites were collected by opening worker sealed brood of *A. mellifera* at the Rural Development Administration (RDA), Suwon, Republic of Korea in November 2012 and October 2013, and the apiary of the College of Agriculture and Life Science (CALS), Seoul National University (SNU), Seoul November to March 2015. All specimens were preserved in 70% ethanol and studied in the Department of Agricultural Biology, National Academy of Agriculture Science (NAAS), RDA, and CALS, SNU, Republic of Korea.

Scanning Electron Microscopy

Fifteen adult female K-type *V. destructor* mites were fixed overnight at 4°C in Karnovsky's fixative (25% glutaraldehyde and 8% paraformaldehyde in 0.2 M cacodylate buffer pH 7.2). Samples were then washed three times in distilled water and fixed in 1% of osmium tetroxide for 2 hours at 4°C All specimens were washed again in distilled water and dehydrated in a graded ethanol series (50, 75, 90, 95, and 100%) for 20 minutes. After dehydration, the samples were dried in a critical point dryer, mounted on SEM stubs, and coated with gold. The specimens were examined with a SEM (Hitachi S2460N, Japan) at 20 kV accelerating voltages.

Morphological measurements

Dorsal and ventral body lengths were measured from the anterior margin to the posterior end of the body. Dorsal and ventral body widths were measured in the middle of the body from one margin to the other. Length and width of sternal, genital, endopodal, metapodal, anal shields, and pedipals were measured. Peritreme length was measured from the distal to proximal ends of the tube. Chelicerae teeth were measured from the base to the tip. Gnathosoma length was measured from the tip of the palps to the basal margin of the subcapitulum. Gnathosoma width was measured at the widest section of the subcapitulum and the length of the legs was measured from their outer margin.

Amplification of mitochondrial COI gene

DNA of K-type *V. destructor* was extracted from the whole body of one mite by the standard proteinase K method (Kocher *et al*., 1989). A partial COI gene (472 base pairs) was amplified by PCR using primer CI-J-1751 ('5-GGAGCTCCTGACATAGCATTCCC-3') and CI-N-2191 ('5-CCCGGTAAAATTAAAATATAAACTTC-3') (Simon *et al*., 1994). PCR conditions were as follows: an initial denaturation at 94°C for 5 minutes, 40 cycles of 94°C for 30 seconds, 50°C for 40 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. To confirm the successful DNA amplification, electrophoresis was carried out using 0.5 X TAE buffer in 1% of agarose gel. The PCR product was then purified using a PCR purification Kit (Qiagen, Germany). DNA sequencing was performed using an ABI 310 Genetic Analyzer (PE Applied Biosystems, USA). Each strand was sequenced twice for accuracy. The sequence alignment 472 nucleotides of the COI fragment was aligned with COI genes of three references *V. destructor* (GenBank accession number. GQ379060, GQ379059, and GQ379063) and *V. jacobsoni* (GenBank accession number GQ387679).

Data Analysis

The means of dorsal body setae lengths, hypostomal setae, *C1*, *C2*, and *C3*, lengths and anal setae, *an1*, *an2* and *an3*, lengths were compared by covariance analysis (one-way ANOVA) to determine any significant differences in lengths. The mean of dorsal and ventral body lengths and widths, lengths of chelicerae teeth (*t1* and *t2*), and apotele claws (*ap.c1* and *ap.c2*) were compared using independent t-tests.

RESULTS

Body sizes

The dorsal shield of female K-type *V. destructor* is ellipsoid, orange-red (Fig. 1A), and the marginal area densely sclerioted. The dorsal shield is 1193.1±35.3μm long (n=18) and 1601.2±73.4μm wide (n=18), the ventral shield is 1233.6±9.0μm long (n=20) and 1233.7±9.2μm wide (n=18) (Fig. 1B). An independent t-test revealed that there were significant differences between the means of dorsal and ventral body lengths (t=4.966, df=36, P<0.0001) and widths (t=2.737, df=36, P<0.01).

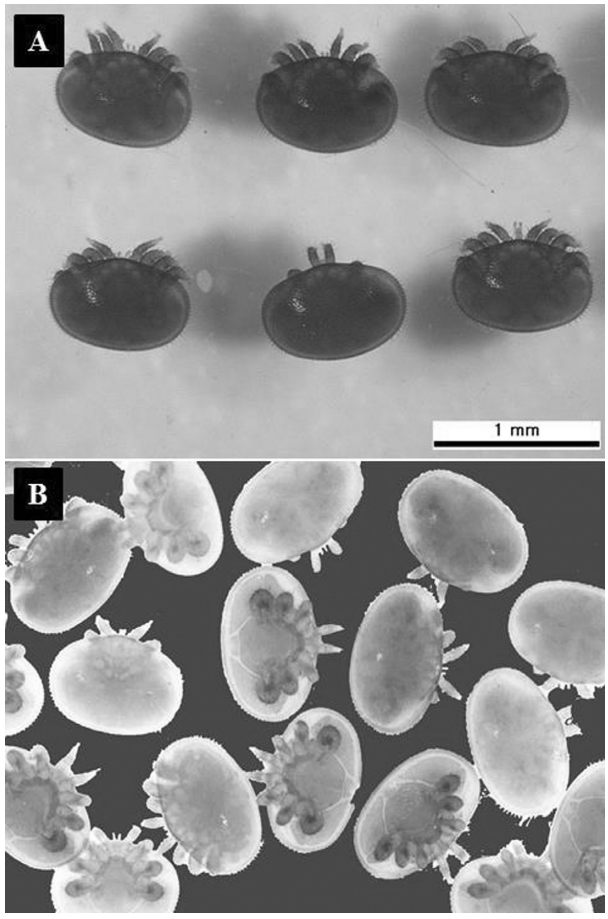

Fig. 1. Morphology of K-type *Varroa destructor*. Dorsal view (A) and Ventral view (B). There were significant differences between the means of dorsal and ventral body lengths (t-test, P<0.01).

Body setae

There are five types of body setae, four with different sizes on the dorsum and one the same size on the venter which project backwards (Fig. 2A). The dorso-anterior setae is short, 51.8±8.4μm long (range 36.7-71.6μm, n=45) (Fig. 2B), the dorso-posterior setae are 112.6±8.0μm long (n=10), i.e. longer than the dorso-anterior ones (Fig. 2C), but shorter than the lateral setae. The dorso-lateral setae are 143.7±25.1μm long (range 103.1-194.6μm) (Fig. 2D). A one-way ANOVA test revealed

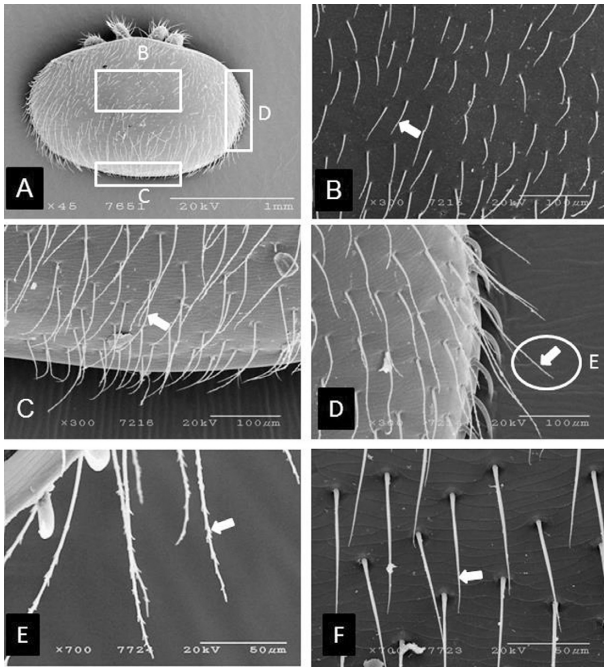

Fig. 2. Scanning electron micrographs of different kinds of body setae of K-type *Varroa destructor*. (A) dorsal setae in different dorsal body parts, (B) higher magnification images of mid-dorsal setae, (C) higher magnification image of posterior setae, (D) lateral setae, (E) higher magnification image of serrated body setae, and (F) ventral setae (simple and barbate). Arrows indicate types of setae.

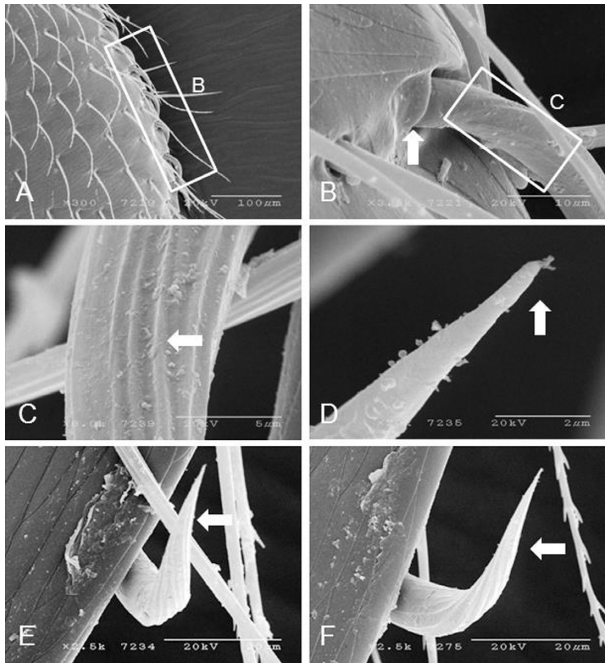

Fig. 3. Scanning electron micrographs of marginal setae of K-type *Varroa destructor*. (A) marginal setae on the lateral side of the dorsal shield, (B) articulating socket, (C) longitudinal ribs on shaft, (D) tip of marginal smooth with ribs, and (E and F) tips of marginal setae showing bending.

that there are significant differences in dorsal seta sizes (ANOVA $F_{(2,75)}$ = 287.5, p=.000). The ventral body setae are 79.2±5.6μm long (range 71.7-87.3μm, n=17). All dorsal setae are serrate (Fig. 2E), whereas the ventral setae are entire, the same size, and barbate (Fig. 2F).

Marginal setae

Marginal setae on both sides of the body originate from the dorsal side of the body (Fig. 3A), which has an individual socket (Fig. 3B). The marginal setae is basally ribbed (Fig. 3C), and half way to the proximal end are smooth and pointed (Fig. 3D) and flexible (Fig. 3E and F). The number of marginal setae is 20.5±1.9 (range 17-24, n=36). The marginal setae is 36.9±2.7μm long (range 31.9-42.0μm, n=19) and 3.8±0.4μm wide (range 2.1-4.5μm, n=19). The distance between each marginal setum is 33.1±10.1μm (range 21.6-52.4μm, n=19).

Ventral shields

Ventral shields are light brown, surrounded by longitudinal muscle, and includes five shields (Fig. 4A). The sternal shield is 487.3±70.9μm long (range 430.1-578.5μm, n=6) and 189.7±7.8μm wide (range 179.4-200.1μm, n=6), densely sclerotized, lack a sternal pore and have 6 pairs of setae with an average length of 101.2±15.2μm (n=23) (Fig. 4B). The genital shield is 672.5±14.9μm long (range 657.6-687.5μm, n=8), and 747.6±8.9μm wide (range 737.3-761.6μm, n=8). The anterior and posterior margins are almost rounded with 105.25±1.3 genital setae. The genital setae is 34.65±3.2μm long (range 29.7-40.6μm, n=13) (Fig. 4C). The endopodal shield is 210.01+0.0μm long, 21.0±0.1μm wide, adjacent to coxa-IV, and with 7 setae. The average length of the endopodal setae is 73.8±14.2μm long (Fig. 4D). The metapodal shield is 355.5±14.3μm long, 144.3±10.9μm wide, broadly triangular, not fused with the genital, endopodal, or anal shields; and with an average of 80.6±6.12 metapodal setae. The metapodal setae is of two types, short and long. The marginal metapodal setae is longer (100.0±10.5μm) than middle metapodal setae (64.7±6.1μm) (Fig. 4E). The anal shield is 153.8±8.5μm long

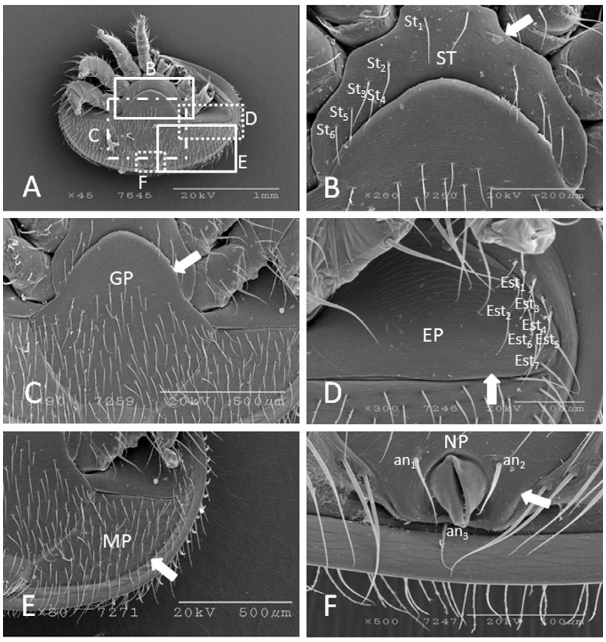

Fig. 4. Scanning electron micrographs of ventral shields of K-type *Varroa destructor*. (A) ventral shields, (B) sternal shield enlarged, (C) genital shield, (D) endopodal shield, (E) metapodal shield, and (F) anal shield. Arrows indicate specific areas of the shields. SP=sternal plate, GP=genital plate, P=endopodal plate, MP=metapodal plate, NP=anal plate, St=sternal setae, Est=endopodal setae, $an_{1\text{-}3}$=anal setae.

(range 144.5-161.4μm, n=5), 259.1±16.9μm wide (range 239.2-279.9μm, n=5), separated from the genital plate and metapodal shield, centrally located, triangular, surrounded by three different sizes of anal setae: an1 (69.1±0.2μm long), an2 (54.4±2.4μm long) and an3 (45.6±7.0μm long). A one-way ANOVA analysis revealed that there is a significant difference in the length of these three kinds of anal setae (F =15.247, df=2, p=.027). A Turkey's post hoc tests revealed that an3 is shorter (Sig.=.024) than an_1 and an_2 (Sig=.083). The para-anal setae (an_1 and an_2) are close to the anus, whereas the post-anal seta (an_3) is posterior to the anus (Fig. 4F). The anal opening extends outside through the anal plate, which is bounded laterally by tiny hinged plates.

Stigma and peritreme

The peritreme is 195.7±8.9μm long (range 184.7-208.8μm, n=5), 7.4±1.6μm wide (range 5.6-9.4μm, n=5), inverted J-shape, covered by the peritremal shield, and extends up to leg-IV (Fig. 5A and B). The tip of the peritreme

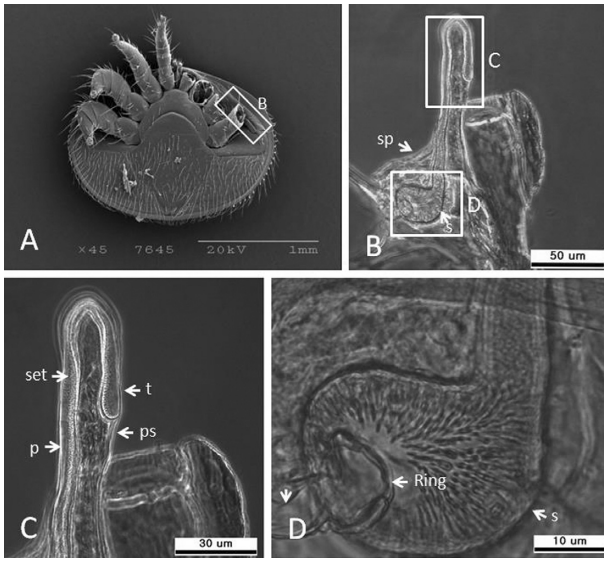

Fig. 5. Peritreme and stigma of K-type *Varroa destructor*. (A) SEM image of the peritreme, (B) peritreme and stigma under the compound microscope (200X), (C) magnification of peritreme bulb (400X), and (D) higher magnification of the stigma (1000X). per=peritreme, ps=peritremal shield, stg=stigma, sp=stigma plate, set=setules. S=stigma.

is bulbous (Fig. 5C). The stigmata 767.0±78.1μm^2(range 698.8-852.2μm^2, n=3) is adjacent to coxae-III and IV, and is surrounded by the stigmal shield (Fig. 5D).

Gnathosoma

The gnathosoma is 312.6±14.5μm long (range 297.1±329.4μm, n=4), 138.3±5.1μm wide (range 134.2-145.8μm, n=4), located antero-ventrally between coxa-I, and semi-concealed under the dorsal shield. The hypostome has 3 pairs of hypostomal setae *viz*, C_1, C_2, and C_3, which are smooth and spiniform (Figs. 6A and B). C_1 and C_3 are longer than C_2, while C_1 and C_2 are close together, but C_3 is separated. C_3 is located near coxa-I. The length of C_1 is 20.5±4.9μm, C_2 13.1±2.2μm and C_3 27.2±0.5μm. A one-way ANOVA analysis revealed that there are statistically significant differences in the lengths of these hypostomal setae ($F_{(2,11)}$=20.606, p=.001). The tritosternum is 128.7±0.4μm long (range 128.4-129.1μm, n=4), well-developed, basally broad, with 6 pairs of barbs on the lateral side (Fig. 6C) and 11 barbs basally (Fig. 6D). The laciniae are barbed and bifid (Fig. 6E). The chelicerae are three-segmented and bear two teeth, t_1 (mean=1.32±0.1μm long, n=4) and t_2 (mean=1.31±0.2μm long, n=4)

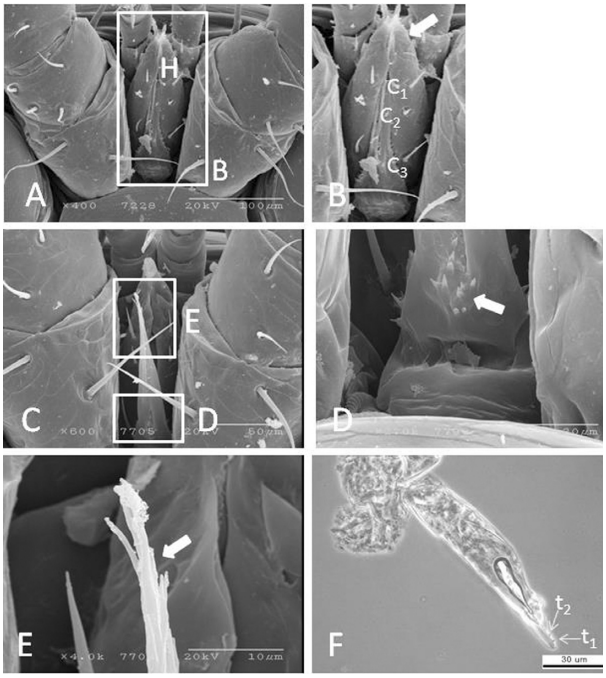

Fig. 6. Scanning electron micrographs of gnathosoma of K-type *Varroa destructor*. (A) hypostome, (B) hypostomal setae, (C) tritosternum, (D) basal of tritosternum showing teeth, (E) tip of tritosternum under the compound microscope, and (F) cheliceral digit with teeth under the compound microscope (400X). H=hypostoma, *h*=hypostomal setae, T=tritosternum, C_{1-3}=hypostomal setae.

(Fig. 6F). An independent t-test failed to reveal any significant differences between the sizes of t_1 and t_2 (t=.045, df=6, p=.965). The distance between t_1 and t_2 is 2.6±0.2μm, (range 2.45-2.98μm, n=4), and the tip of fixed digital is slightly curved and pointed.

Palptarsi

The pedipalps are 179.4±9.7μm long (range 167.3-192.2μm, n=5) and five-segmented (Fig. 7A). The pedipal-tarsi have long and short apotele claws (ap.c_1 and ap.c_2). The ap.c_1 is 37.6±3.1μm long and ap.c_2 is 10.1±1.9μm. (Figs. 7B and C). An independent t-test revealed that there is a significant difference in the lengths of ap.c_1 and ap.c_2 (t=22.690, df=17, P=.001). The ap.c_2 is inserted on the base of ap.c_1. The pedipal-tarsi have 16 sensory setae (Fig. 7D). Pedipal setation: trochanter 1, femur 1, genu 1, tibia 5, and tarsi 16 setae. The palp-femural has only one seta 70.71μm long, which is longer than the gena seta (54.45μm).

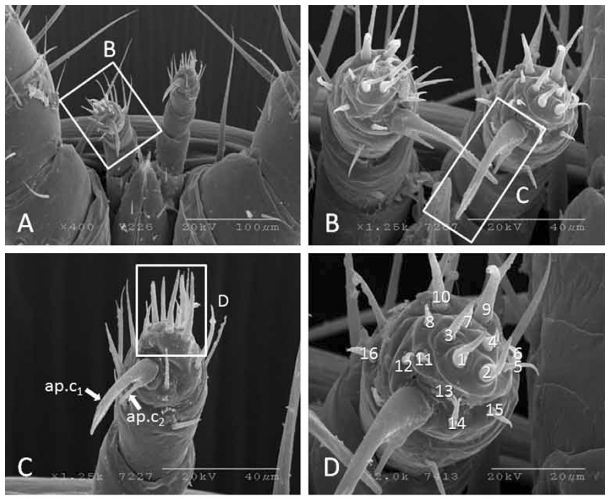

Fig. 7. Scanning electron micrographs of palptarsi of K-type *Varroa destructor*. (A) pedipals, (B) palpal apotele claws, (C) higher magnification of claws, and (D) higher magnification of palpal-tarsus showing palpal setae arrangement. Note: Roman numbers indicate sensory setae on palptarsi. ap.c_1=long claw, ap.c_2=short claw.

Leg

The legs have varied lengths. Leg-I is 624.4μm long, somewhat rounded, and the condylophore is 78.94μm long (Fig. 8A). Leg-II is 584.3μm long and dorso-ventrally flat with a short condylophore (Fig. 8B). Leg-III is 812.8μm long, completely flattened, with short tarsi and the condylophore is 100.68μm long (Fig. 8C). Leg-IV is 970.3μm long, completely flattened, and the condylophore is 86.07μm long (Fig. 8D). Leg setae are smooth, simple, and spiniform. The tarsi of leg-I-IV have ambulacral sucker-like structures which are used for walking and attachment. Leg setation: coxae 2-2-2-2, tronchanters 5-6-6-6, femur 6-9-8-6, genu 4-8-11-10, tibia 8-6-8-8, and tarsus 29-18-20-20.

COI gene sequence

The COI gene of mtDNA of K-type *V. destructor* is closely related to GQ379060 (100% nucleotide sequence identity), GQ379059 (99.59% nucleotide sequence identity), and GQ379063 (98.78% nucleotide sequence identity) GenBank *V. destructor* (Fig. 9).

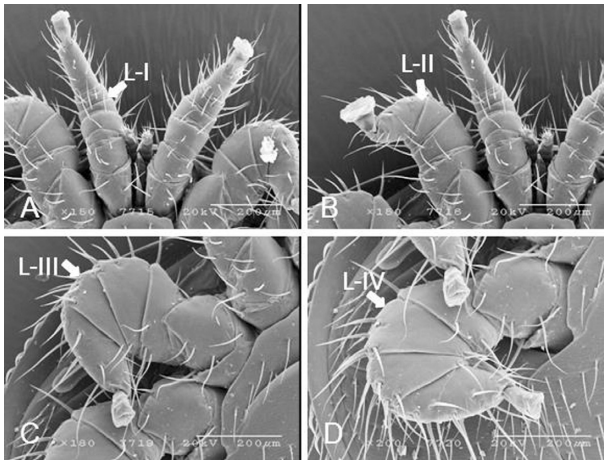

Fig. 8. Scanning electron micrographs of legs of K-type *Varroa destructor*. Legs were categorized by length as Leg I, Leg II, Leg III, and Leg IV. (A) Leg-I is 624.4μm long and rounded. The condylophore is 78.94μm long. (B) Leg-II is 584.3μm long and dorso-ventrally flat with a short condylophore. (C) Leg-III is 812.8μm long, completely flattened, with short tarsi and the condylophore is 100.68μm long. (D) Leg-IV is 970.3μm long, completely flattened, and the condylophore is 86.07μm long.

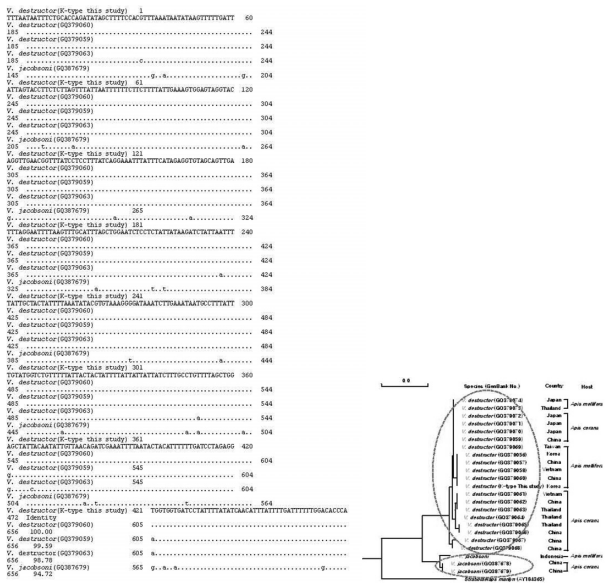

Fig. 9. Multiple alignment of mitochondrial COI gene (472bp) of the K-type *V. destructor* with known GenBank *Varroa* mite mitochondrial COI genes. Identical nucleotide sequences are dotted. K-type *V. destructor* was closely related to GQ379060 (100%), GQ379059 (99.59%), and GQ379063 (98.78%) of GenBank accession number of *V. destructor* cox1 genes.

DISCUSSION

Body size

Our results showed that K-type *V. destructor* is longer than Japan/Thai-Vietnam types including Luzon haplotype-1 and 2 in length, but smaller than Japan/Thai-Vietnam and New Zealand types in width (Table 1). This indicates that K-type *V. destructor* is oval rather than rounded (Japan/Thai-Vietnam types) (Table 1). The difference may be due to geographical variation. The K-type *V. destructor* is broader, but shorten than Japan/Thai Vietnam type of *V. destructor* (Anderson and Trueman, 2000) and the New Zealand type of *V. destructor* (Zhang, 2000) (Table 1) where the author reported that the *V. destructor* of New Zealand (Auckland) type is similar to the Japan/Thailand-Vietnam type. This indicates that the Japan and Korean haplotypes have different morphological features. Both haplotypes are distinguishable only by differences in mtDNA sequences (Anderson and Trueman, 2000).

Body setae

SEM micrographs showed dorsally serrated setae dorsally and entire simple setae ventrally. The most possible explanation is that the dorsal setae are probably used to interlock with the body hairs of bees during the phoretic period. The ventrally entire spinescent setae may help *V. destructor* to walk on brood cell and on bee's bodies. All these setae are reflexed posteriorly.

Marginal setae

SEM micrographs of the marginal setae showed that the tips are smooth and flexible. The morphological plasticity suggest that K-type *V. destructor* also uses the marginal setae to grab the integument of the larvae during the feeding periods inside the sealed cells, and also use to grap the adult bees during hibernation times inside the tergits of winter bees, and the phoretic period. Davis and Camin (1976) have demonstrated that the primary role of marginal setae in *Dermanyssus prognephilias* mites, ectoparasites of birds, is for attachment.

Table 1. Body lengths and widths of female K-type *V. destructor* compared with those of *V. destructor* reported by Anderson and Trueman (2000) and Zhang (2000)

Species	*Varroa* types	Body length (μm)		Body width (μm)		n
		Mean	SD	Mean	SD	
V. destructor	Korean haplotype***	1193.1	35.3	1601.2	73.4	18
V. destructor	Luzon 1 haplotype*	1055.2	15.6	1526.2	25.7	5
V. destructor	Luzon 2 haplotype*	1067.4	21.4	1524.0	27.8	21
V. destructor	Mindanao haplotype *	1086.0	22.4	1616.5	26.7	14
V. destructor	Japan/Thailand-Vietnam*	1167.3	26.8	1708.9	41.2	533
V. destructor	New Zealand type**	1159.0	21.6	1700.0	46.5	5

*Anderson and Trueman (2000), **Zhang 2000, *** *V. destructor* used in this study.

Table 2. Some morphological features of female K-type *V. destructor* compared with two other *Varroa* species reported by de Guzman and Rinderer (1999)

Character	K-type *V. destructor*		*V. jacobsoni*		*V. rindereri*	
	Mean	SD	Mean	SD	Mean	SD
Body length (μm)	1193.1	35.3	1077	6	1180	11
Body width (μm)	1601.2	73.4	1596	10	1698	14
Peritreme length (μm)	195.7	8.9	426	9	582	13
Loop distance			18	2	49	2
Number of marginal setae	20.5	1.9	19	0.4	23	0.5
Number of endopodal seate	7.0	0.0	7	0.3	12	0.5
Number of sternal setae	6.0	0.0	10	0.3	9	0.3
Number of sternal pores	0.0	0.0	11	0.4	9.0	0.4
Number of metapodal setae	80.6	6.1	22	0.5	23	0.8

Ventral shields

The sternal shield has a fixed number of sternal setae. The *St3* and *St4* are adjacent, whereas other sternal setae separated and asymmetric. SEM micrographs indicate that the endopodal shield has a fixed number of endopodal setae and no marginal setae. These are diagnostic characters of K-type *V. destructor*. The number of endopodal setae of K-type *V. destructor* is similar to *V. jacobsoni*, but less than *V. rindereri* (Table 2). A comparison of endopodal setae needs to be made with *V. destructor* reported from other parts of the world. The metapodal shield has two types of setae, long and short. The long setae protrude ventrally outside of the body margin and are used to protect the longitudinal muscles running along the outer margin of the body from rubbing. These setae may also assist in locomotion. The number of metapodal setae in K-type *V. destrcutor* is more than *V. jacobsoni* and *V. rendereri*. In contrast, the number of marginal setae of K-type *V. destructor* is very close to *V. jacobsoni*, but less than *V. rindereri* (Table 2).

Stigma and peritreme

Our observations showed that one end of the peritreme opens into the stigma and the other end is a bulb-like structure without an opening. The peritreme groove is internally lined with setules and micropapillae (Bruce *et al*., 1997). The peritreme of K-type *V. destructor* is smaller than *V. jacobsoni* and *V. rindereri* (Table 2). The stigma has an opening indicated by a ring-like structure (Fig. 4D). We are doubtful that air reaches into the atrium directly through the peritreme. Our observations did not support the theory that the air comes from directly through the peritreme because the tip of the peritreme is closed, covered by peritreme sheath, and flexible. It seems likely that the peritremes may be used to move air into the atrium through the stigma opening. More observations are needed to clarify the functions of peritremes.

Table 3. Leg chaetotaxies of female K-type *V. destructor* compared with female *V. jacobsoni* (Akaratanakul, 1975)

Varroa species	Leg	Coxa	Trochanter	Femur	Genu	Tibia	Tarsus
K-type	I	2	5	6	4	8	29
V. destructor	II	2	6	9	8	6	18
	III	2	6	8	11	8	20
	IV	2	6	6	10	8	20
*V. jacobsoni**	I	2	5	11	12	13	NR
	II	2	5	10	11	11	NR
	III	2	5	8	11	11	NR
	IV	1	6	7	9-11	10-11	NR

*Akaratanakul, (1975). "NR"± not reported.

Gnathosoma and palptarsi

SEM micrographs showed that *V. destructor* has two apotele claws, long and short, originating from a single base at the palptarsus. The tip of the long apotele claw is pointed and slightly incurved, whereas the tip of the small apotele claw is blunt and straight. The structure of the long apotele claw tip indicates that they are used to pierce the integument of adult bees and larvae. The short apotele claw with a rounded tip has no piercing function, but probably helps to grip the integument during feeding and phoretic periods. SEM micrographs showed that the palptarsi have 16 sensory setae at different layers. These may function as the sensilla on the palptarsus of female *V. jacobsoni* which considered to be chemoreceptors to perceive chemicals in drone cells (Liu, 1988; Liu and Peng, 1990).

Legs

SEM micrographs showed that leg I is more rounded compared to legs II, III and IV. The most plausible explanation is that *V. destructor*, due to the presence of mechano-chemoreceptor sensory setae on leg I, rarely uses for walking. Legs II-IV are dorso-ventrally flattened with pairs of short and long spiny setae are used for walking. The short spiny setae on the lateral side of legs III and IV indicate that these setae may protect the mite from bee grooming. *V. destructor* has two types of setae, long and short, compared with *V. jacobsoni*, which has only one type of leg with long setae (Akratanakul, 1975). The leg chaetotaxis of K-type *V. destructor* has some differences in the number of setae on particular segments (Table 3).

Conclusion

The K-type of *V. destructor* has more oval shape with three distinct types of body setae, viz serrated type dorsally, barbs type ventrally and smooth type setae on the body margins. The anal opening is protruded and semi-bounded by tiny hinged plates from the top of opening. Cheliceral bear two teeth of similar size teeth. The apotele claws are short and long types which may use to pinch the integument of developing larvae and adult honey bees during the feeding time. The body shape, seate, and cheliceral morphology is central to the evolutionary success of survival of *V. destructor* mites.

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