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UV-C radiation-induced changes in the legs and trichoid sensilla of the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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Abstract: Since various kind of receptors in insects are mainly located on their legs. We estimate the influence of ultraviolet-C on the leg morphology and trichoid sensilla of *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae), zero-day-old pupae were irradiated with UV-C at a distance of 35 cm for 1, 2, 4, 8, 16, 32, and 64 min. Changes in leg morphologies and trichoid sensilla were determined using light and scanning electron microscopes. UV-C radiation caused the abnormalities of an adult's legs that were classified into three levels; mild, moderate, and strong. Legs of the resulting adults were developed into malformed structures that lacked clear segmental identity. The severity of leg abnormalities was recorded in a dose-dependent manner. UV-C significantly decreased the length and width of the femur, tibia, and tarsus. In the strongly affected level, the femur was reduced in size while the tibia and tarsus were completely deleted, and there was no evidence of claws. Scanning electron microscopy showed the wrinkled cuticle on the leg segment, and the sensilla of the resulting adults appeared less in number. After UV-C radiation, the trichoid sensilla on the femur became longer and wider, but the trichoid sensilla on the tibia were shorter and narrower. Taken together, UV-C irradiation during pupal stage interfered development of legs and brought about specific malformation features.

Key words: UV-C, radiation, red flour beetle, legs, sensilla

Introduction

Tribolium castaneum (Herbst, 1797) (Coleoptera: Tenebrionidae) is a common pest of stored grains, particularly of wheat flour and rice bran, and is an important factor in reducing the quality of these foods. The adult beetles contain scent glands that secrete malodorous fluid, which causes a bad smell to infested the material (Lu *et al*. 2011).

The *T. castaneum* adult legs consist of six different segments: the coxa, trochanter, femur, tibia, tarsus, and pretarsus (Smith *et al*. 2014). Insect chemical communications are based on receptors that are mainly located on the legs. Receptors involved in feeding preferences, recognise food odors and play key roles in insect survival and adaptation to environmental changes (Skiri *et al*. 2005, De Boer 2006). Multiple receptors provide several functional advantages to an insect's ability to perceive and respond to stimuli (Debry & Steullet 2001). Trichoid sensilla have an olfactory function (Hallberg *et al*. 2003) and trichoid sensilla on the tibia of *T. castaneum* share many features typical of mechanosensory hair (Seada & Hamza 2018). Generally, chemosensory associated proteins such as odorant binding proteins, chemosensory proteins and gustatory receptors are usually expressed in insect legs (Li *et al*. 2020).

The ultraviolet (UV) portion of the spectrum has been used to kill the germs and for the disinfestation of insects in different stages of the life cycle (Baden *et al*. 1996). Among these, UV-C radiation has been reported to influence biological systems in various organisms (Espo *et al*. 2015). Our previous study showed that UV-C radiation caused severe abnormalities on the elytra, hindwing, antennae, and altered the antennal sensilla of *T. castaneum*

(Tungjitwitayakul *et al*. 2019, 2020). Several studies have reported that UV-C radiation could cause abnormalities in the insect appendages. In addition to UV-C, gamma irradiation also has a clear deformity effect on the leg parts of the cotton leafworm, *Spodoptera littoralis* (Boisduval, 1833), specifically pretarsus, tarsomere, tarsal segment, and spurs (El-Degwi & Gabarty 2015). When 4th-instar larvae and pupae of alfalfa weevils, *Hypera postica* (Gyllenhal, 1813), were irradiated with gamma-ray, the resulting adults had abnormal antennae, legs, and elytra (Burges & Bennett 1971, 1972).

In the present study, changes in the morphology of the legs of *T. castaneum* were determined after UV-C radiation during the pupal stage. The abnormalities of the resulting adults were recorded, and the shape and size of the legs were measured. In addition, the surface morphology of the legs was investigated using scanning electron microscopy (SEM) to determine the effect of UV-C on the ultrastructure of *T. castaneum* legs. The length and width of trichoid sensilla on the femur and tibia were also measured to evaluate the impact of UV-C on the sensilla structure. Taken together, our research demonstrate that the UV-C radiation clearly affects leg morphology and trichoid sensilla structure of *T. castaneum* that lead to provide information necessary for pest management.

Materials and methods

Beetle culture

Tribolium castaneum was cultured on sterile whole wheat flour mixed with 5% yeast (Suzuki *et al*. 2009). The containers were kept at 2±28˚C and relative humidity of 65±5% and light/dark photoperiod of 16/8 h. Under this rearing environment, eclosion of adults occurs 7 days after pupation.

UV-C radiation

The radiation was performed in a test chamber (90 × 60 × 55 cm^3). The lamp, which was used as the radiation source emitting radiation at a wavelength of 254 nm (17-watt UV germicidal lamp (TUV F17T8, Philips, Amsterdam, The Netherlands) measuring 58 × 2.5 cm^2) was fixed to the ceiling of the chamber. The stage on which the insects were exposed to radiation was 35 cm from the surface of the UV lamp. During pupal stage, dramatic tissue reorganization occurs and adult structures develop (Suzuki *et al.* 2009). Then 0-day-old pupae were selected to examined the effect of UV-C. The dorsal sides of 0-day-old pupae were fixed to a glass slide using double-sided adhesive tape. The slides with mouthed pupae were exposed to UV-C radiation for 0, 1, 2, 4, 8, 16, 32, and 64 min and doses of UV-C in each exposure time were measured and calculated as 0.2, 0.8, 3.2, 12.8, 51.2, 204.8, and 819.2 W/cm^2, respectively. Three replicates of each treatment were done using 20 pupae. After UV-C irradiation, the pupae were transferred from the slides using a fine paintbrush and kept at the rearing condition as mentioned in beetle culture for 7 days or until adult eclosion.

Preparation of leg specimens

Beetles were immobilised on ice and then fixed in 70% ethanol. Their leg specimens were carefully dissected with a pair of fine forceps and scissors under a stereo microscope.

Documentation and image processing

The dissected legs of *T. castaneum* were observed under an Olympus SZ51 stereomicroscope (Olympus Corporation, Tokyo, Japan) and the images were digitally captured by an Optika microscope (4083 wifi) (OPTIKA Srl, Ponteranica, Italy). Some images were documented by Adobe Photoshop CS3 to enhance only brightness and contrast.

Leg measurements

The length of the individual leg segments

(coxa, trochanter, femur, tibia, and tarsus) was measured along the proximal-distal axes, and the width of the coxa, femur, tibia, and the 3rd segment of tarsus was measured using Adobe Photoshop CS3 software.

Scanning electron microscopy (SEM)

Samples were collected from 7-day-old adult insects in the control group and UV-C-treated group. The leg specimens were collected from dried adults at 60˚C for 24 h in a hot air oven. The dried specimens were bound to a copper stage with adhesive tape, and then the surface of the specimen was covered with gold particles using an ion sputtering instrument. The coated specimen was observed with a JSM-6610LV scanning electron microscope (JEOL, MA, USA).

Sensilla measurements

The SEM images were observed using Adobe Photoshop CS3 to examine the sensilla size. The length of the individual trichoid sensilla on the femur and tibia was measured along the proximal-distal axes, and the width at its middle part was measured.

Data analysis

A statistical analysis of the percentage of leg abnormalities and the length and width of legs were performed using a one-way analysis of variance (ANOVA), followed by a least-significance-difference (LSD) multiple-range test. The length and width of trichoid sensilla between control and UV-C irradiated group were analysed using paired samples T-tests (IBM SPSS Statistics 22). The significance level was set at 0.05 ($P<0.05$).

Results

Effect of UV-C radiation on leg abnormalities

UV-C caused several abnormalities in the legs of *T. castaneum* (Fig. 1). These abnormalities were classified into three levels; mild, moderate, and strong. At the mildly affected level, the coxa, trochanter and femur of forelegs, midlegs, and hindlegs were similar to control, whereas the tibia was reduced in size. The segments of the tarsus are greatly reduced, and loss of normal segmentation patterns. The claws are still present at the tip of the forelegs and midlegs but a loss in hindlegs (Fig. 1B, F, J). At the moderately affected level, the femur appears slightly misshapen (Fig. 1C, G, K). In the hindlegs, the tibia was shortened and dramatic reduction in width (Fig. 1K), whereas the tibia in forelegs and midlegs were observed as a small limb (Fig. 1C, G). The tarsus was completely deleted in the forelegs and midlegs but greatly reduced in hindlegs (Fig. 1C, G, K). In the strongly affected level, the femur segments are shorter and narrower compared to control. The tibia and tarsus in forelegs, midlegs, and hindlegs were lost (Fig. 1D, H, L).

Percentage of leg abnormalities

The severity of leg abnormalities gradually increased with the duration of UV-C radiation (Table 1). At 1 min of radiation, the percentage of abnormalities of forelegs, midlegs, and hindlegs were recorded in the mildly affected level, which presented at 40.00±10.34, 46.48±3.52, and 20.00±5.77%, respectively. At 2 min of radiation, the percentage of mildly affected forelegs, midlegs, and hindlegs was increased. At 4 min of radiation, the moderately affected level was recorded in the forelegs and midlegs with 22.09±2.47 and 14.18±3.76%, respectively. In contrast, the moderately affected level could not be observed in the hindlegs when the insect was exposed to 4 min of radiation. At 8 min of radiation, the strongly affected level was observed only in forlegs with low percentages (10.00±1.92%) and the moderately affected level was recorded in midlegs and hindlegs at 16.85±3.91 and 13.33±3.33, respectively. At 16 min of radiation, the strongly affected forelegs and midlegs were increased to 17.32±4.11 and

Table 1. The percentage of foreleg, midleg and hindleg abnormalities in adult resulting from UV-C radiated pupae for 1-64 min which was classified into mildly, moderately and strongly affected.

Level of abnormality	Radiation time (minutes)							
	control	1	2	4	8	16	32	64
Foreleg								
normal	100.00^{a}	60.00±10.34^{b}	45.94±2.41^{bd}	23.82±7.82^{ce}	28.89±8.89^{cd}	21.65±8.44^{ce}	9.82±1.18^{ef}	0^{f}
mild	0^{a}	40.00±10.34^{bc}	54.06±2.41^{b}	54.09±9.39^{b}	33.33±3.85^{c}	43.07±7.04^{bc}	43.47±6.66^{bc}	31.27±4.72^{c}
moderate	0^{a}	0^{a}	0^{a}	22.09±2.47^{bc}	27.78±4.00^{b}	17.96±2.07^{cd}	12.15±2.92^{de}	6.59±1.71^{e}
strong	0^{a}	0^{a}	0^{a}	0^{a}	10.00±1.92^{ab}	17.32±4.11^{b}	34.56±6.92^{c}	62.14±6.35^{d}
Midleg								
normal	100.00^{a}	53.52±3.52^{b}	34.37±6.49^{c}	23.11±6.59^{cd}	24.47±3.19^{cd}	24.19±1.56^{cd}	15.94±3.99^{d}	0^{e}
mild	0^{a}	46.48±3.52^{bd}	65.63±6.49^{c}	62.71±4.73^{c}	58.68±7.09^{bc}	52.45±3.21^{bcd}	40.43±4.79^{d}	26.41±2.57^{e}
moderate	0^{a}	0^{a}	0^{a}	14.18±3.76^{bc}	16.85±3.90^{b}	9.23±0.77^{c}	10.84±3.41^{bc}	10.37±0.37^{bc}
strong	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	14.13±4.55^{b}	32.79±6.81^{c}	63.22±2.69^{d}
Hindleg								
normal	100.00^{a}	80.00±5.77^{b}	76.67±8.82^{b}	50.00±10.00^{c}	20.00±5.77^{d}	26.67±8.82^{d}	0^{e}	0^{e}
mild	0^{a}	20.00±5.77^{ab}	23.33±8.82^{b}	50.00±10.00^{cd}	66.67±6.67^{c}	46.67±8.82^{cd}	46.67±6.67^{cd}	33.33±3.33^{bd}
moderate	0^{a}	0^{a}	0^{a}	0^{a}	13.33±3.33^{b}	26.66±8.82^{c}	40.00±5.77^{d}	40.00±5.77^{d}
strong	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	13.33±3.33^{b}	26.67±8.82^{c}

Data are shown as the mean ± standard error (SE) (n = 20) followed by the same letter in a row are not significantly different at $P < 0.05$ by LSD's test.

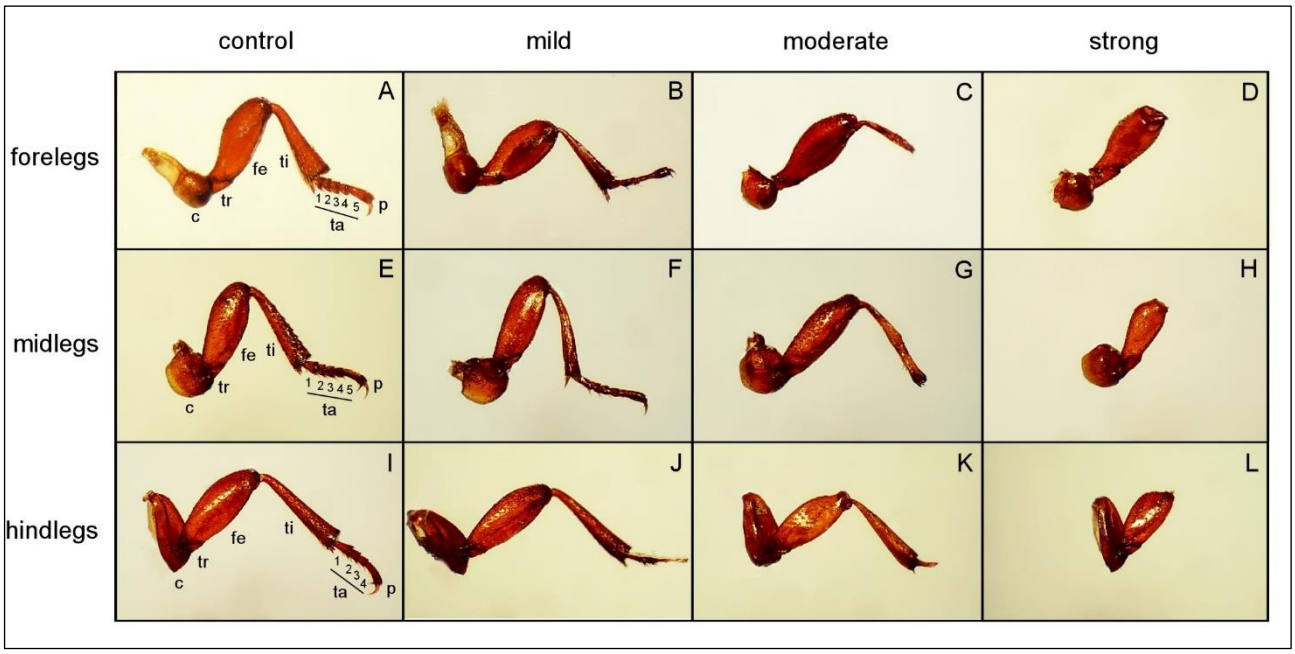

Fig. 1. Morphology of adult legs from control (A, E, I) and UV-C radiated pupae. The abnormalities of forelegs, midlegs, and hindlegs from mildly (B, F, J), moderately (C, G, K), and strongly affected levels (D, H, L) of adults developed from UV-C radiated pupae were shown for comparison. Abbreviations: c, coxa; fe, femur; p, pretarsus; ta, tarsus; ti, tibia; tr, trochanter.

14.13±4.55%, respectively, but the strongly affected level could not be detected in hindlegs. At 32 min of radiation, the percentage of strong abnormality in forelegs and midlegs was increased, and the strongly affected hindlegs were recorded at 13.33±3.33%. As compared to other radiation periods, the percentage of strongly affected

forelegs, midlegs, and hindlegs were highest at 64 min of radiation with 62.14±6.35, 63.22±2.69, and 26.67±8.82%, respectively. More specifically, the adult with normal forelegs, midlegs, and hindlegs could not be detected at 64 min of radiation.

Effect of UV-C radiation on size of legs

We measured the length and width of the forelegs, midlegs, and hindlegs in the resulting adults that were designated as mildly, moderately, and strongly affected levels. UV-C radiation-induced various degrees of leg length and width reduction.

In the forelegs, the strongly affected level had longer coxa than in other groups, including control (Fig. 2A), whereas the width of coxa in the strongly affected forelegs was narrower than control, mildly, and moderately affected level (Fig. 2A, B). UV-C had no effect on the length of the trochanter on forelegs (Fig. 2A). However, the length and width of the femur were dramatically decreased in moderately and strongly affected levels compared to the mildly affected level and control (Fig. 2B). The length and width of the tibia in the moderately affected forelegs were decreased significantly compared to the control and mildly affected levels. Likewise, the lengths and width of the tarsus on the mildly affected forelegs were shorter than those of control (Fig. 2A, B). However, we could not detect the tarsus in the moderately and strongly affected groups.

UV-C radiation could not alter the length and width of coxa in the midlegs but slightly increased the length of the trochanter in mildly and moderately affected levels (Fig. 2C, D). The length and width of the femur and tibia were dramatically decreased in moderately and strongly affected levels. The length of tarsus in mildly affected midlegs become shorter, and the width becomes narrower than the control condition (Fig. 2C, D). More specifically, the tarsus of the midleg in moderately and strongly affected groups was not found in the forelegs.

In the hindlegs, the length of coxa in mildly and moderately affected levels was shorter than those of control, but UV-C had no effect on the width of coxa (Fig. 2E, F). After UV-C radiation, the trochanter becomes longer in length (Fig. 2E). The length and width of the femur in moderately and strongly affected levels decreased significantly compared to the controls. Subsequently, the length and width of the tibia in the strongly affected level were shorter than control and other groups. For the tarsus, the length at the moderately affected level was shorter, and the width in mildly and moderately affected level become narrower than the controls (Fig. 2E, F). The tarsus was not detected in the strongly affected group.

Effect of UV-C radiation on the microstructure of legs

Figure 3 compares the hindlegs of the control and resulting adults in the moderately affected level under SEM. In general, numerous trichod sensilla are scattered homogeneously on the surface of the femur and tibia (Fig. 3C, E). The distal end of the tibia was fringed with strong cuticular spines around the dorsal and lateral sides. There were two ventral tibial processes (tp) devoid of sensilla at the distal end of the tibia (Fig. 3G). The four segments of the tarsus had chaetica sensilla (chs), these sensilla were curved with longitudinal grooves, and their cuticle appeared thick. There were inserted in the cuticle via a depressed membranous base (Fig. 3G).

The femur and tibia of hindlegs in the control showed the smooth surface of the cuticle (Fig. 3C, E), whereas the cuticle of femur and tarsus at the moderately affected level had a rough surface (Fig. 3D, F). The number of trichoid sensilla (ts) on the femur of the resulting adult was less than in the controls, but these sensilla were longer than in the controls. The base of the sensilla was broader, and the basal socket was shallower than control as indicated with a white circle (Fig. 3D). The base of the tibia was narrower (Fig. 3B, F), the tarsus was the loss of normal segmentation patterns, and the claw was

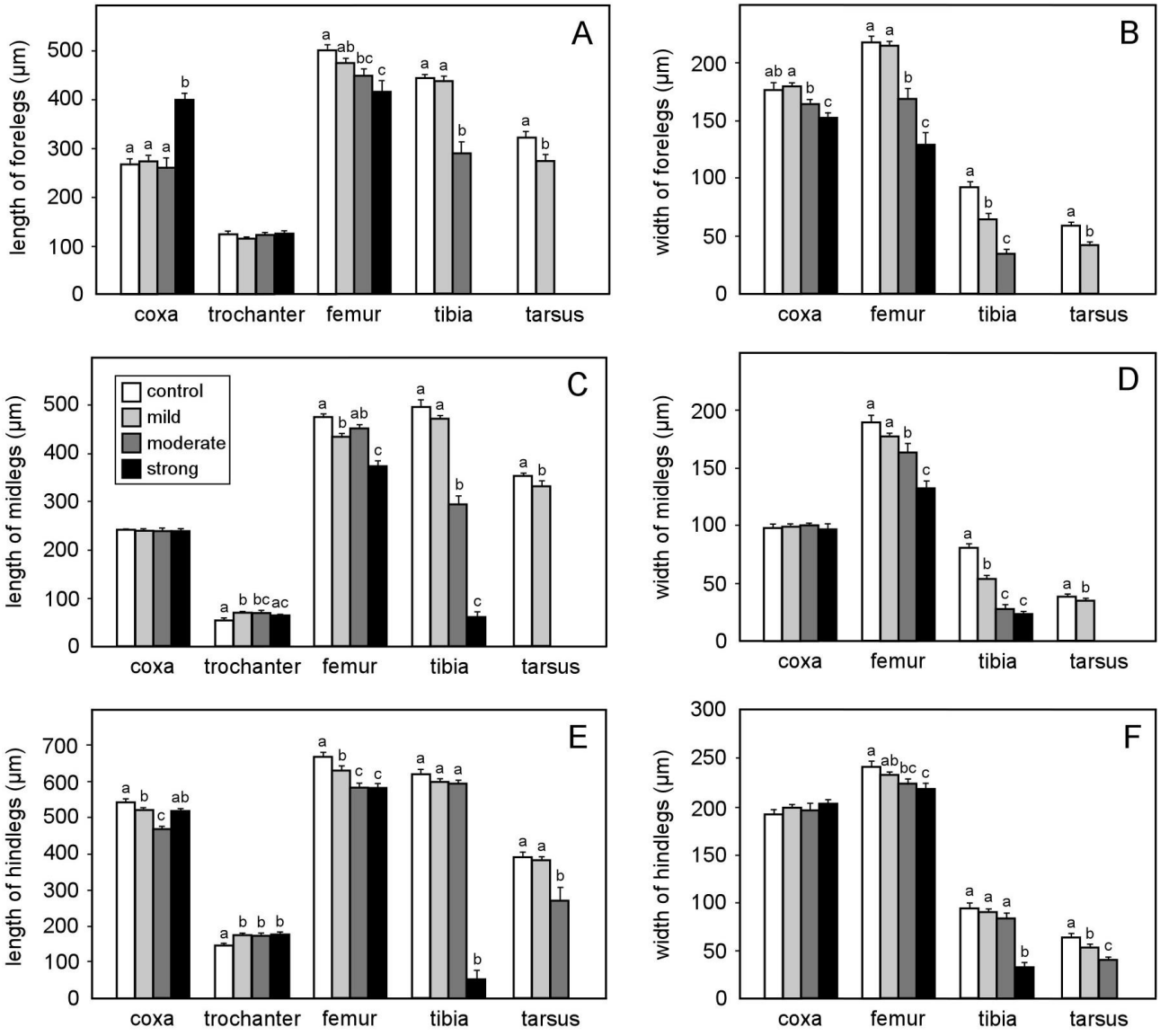

Fig. 2. Analysis of length and width of legs after UV-C radiation. The length (A, C, and E) and width (B, D, and F) of forelegs, midlegs, and hindlegs were compared between control and UV-C-irradiated insects (mildly, moderately, and strongly affected levels). Data are shown as the mean values±SE. The means labeled with the same letters in each leg segment are not significantly different ($P< 0.05$, LSD test, ANOVA).

completely deleted (Fig. 3H). Also, the tibial spines (tis) and tarsal processes (tp) also reduced in size (Fig. 3H).

The deformities of the forelegs are shown in Fig. 4. At the moderately affected level, it clearly shows the malformed femur and tibia. The basal and distal parts of the femur were reduced in size while the tibia was malformed and appeared as a short limb (Fig. 4A–E). At the strongly affected level, there was an indication that the tibia was strongly affected by UV-C radiation compared to other parts of the leg (Fig. 4F). Taken together, UV-C had a greater effect on the distal segment than the basal segment of *T. castaneum* legs.

Effect of UV-C radiation on the trichoid sensilla of legs

Comparison of the length and width of trichoid sensilla between control and resulting adult at the moderately affected level showed that the length and width of trichoid sensilla on the femur of UV-C radiated group was longer

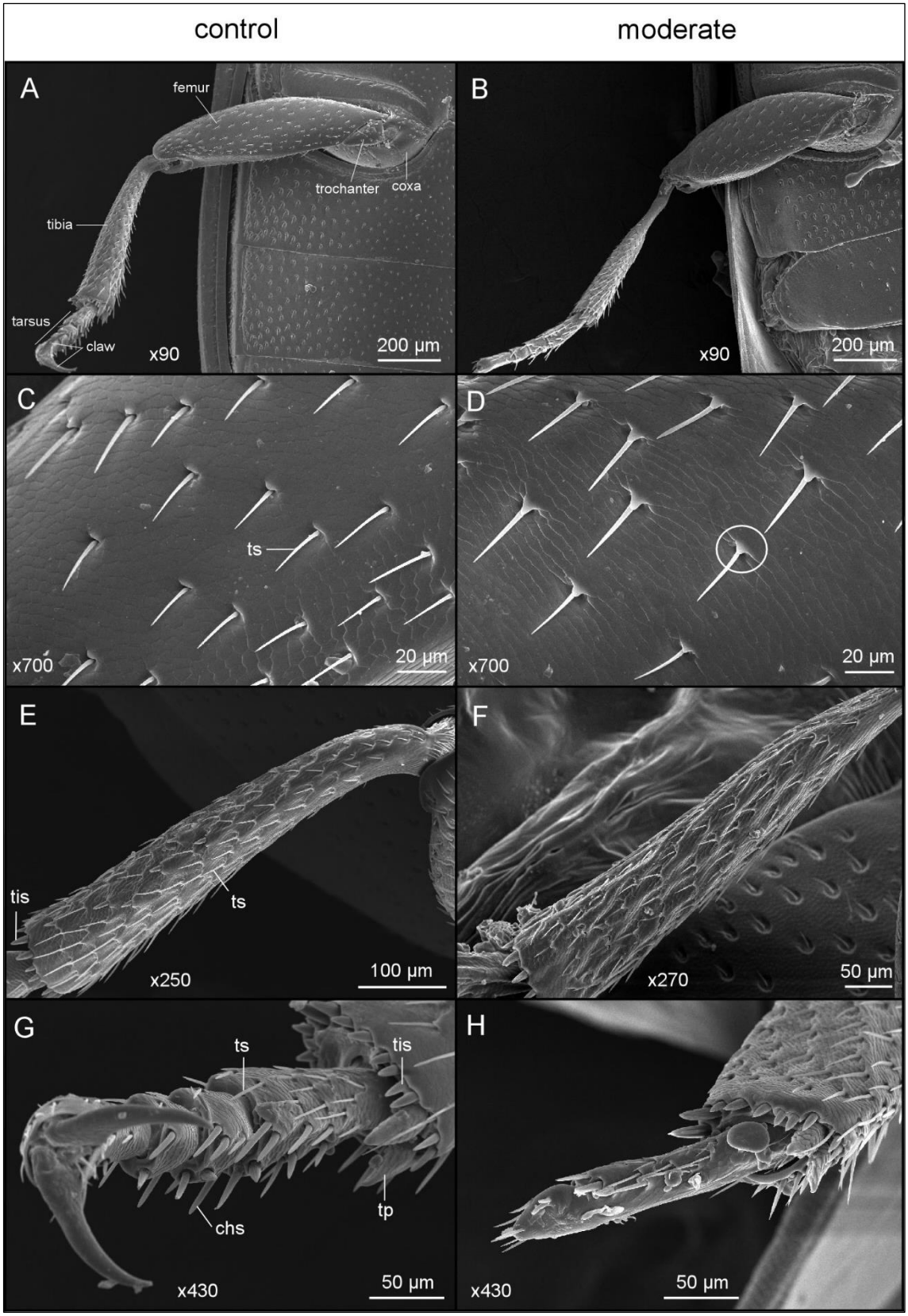

Fig. 3. The morphology of the legs in control and the resulting adults were analysed by scanning electron microscope (SEM). (A) Hindleg of control adult. (B) Hindleg of a moderately affected level adult. (C, E, G) Higher magnification of the femur, tibia, and tarsus-claw in A, respectively. (D, F, H) Higher magnification of the femur, tibia, and tarsus-claw at the moderately affected level, respectively. Abbreviations: chs, chaetica sensilla; ts, trichoid sensilla; tp, tarsal processes; tis, tibial spines.

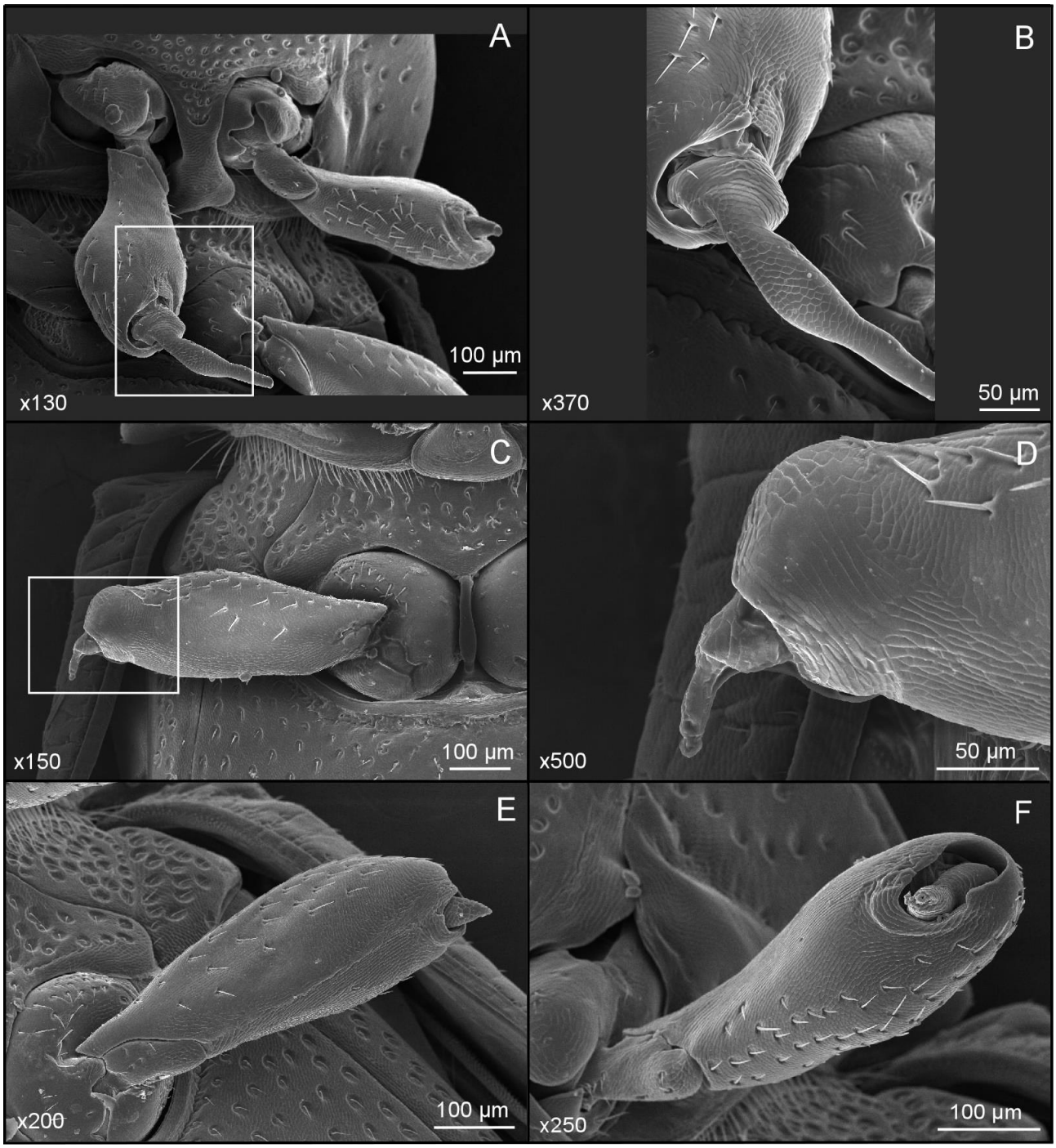

Fig. 4. Scanning electron micrographs (SEM) of the forelegs from the resulting adults of UV-C irradiated pupae. (A-E) The malformed femur and tibia of moderately affected levels. The images of the tibia in B and D are enlargements of the area indicated by white rectangles in A and C, respectively. (F) The malformed femur of strongly affected levels.

relative to the control. In contrast, the length and width of the trichoid sensilla on the tibia in the resulting adult was shorter than the control group (Fig. 5A, B). This is indicating that trichoid sensilla responded to UV-C radiation differently.

Discussion

UV-C radiation on 0-day-old pupae caused malformed legs in the resulting adults of *T. castaneum*, and the levels of abnormalities were dose-dependent. Comparing between

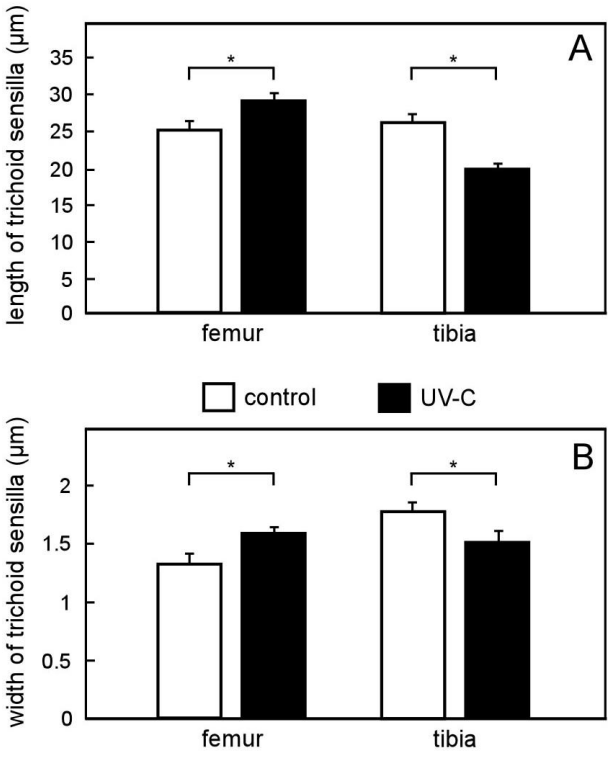

Fig. 5. Comparison of the length (A) and width (B) of trichoid sensilla on the femur and tibia of control and resulting adults (moderately affected level). The length and width of trichoid sensilla were compared with the paired-sample T-test, columns marked with an asterisk are significantly different between the control and UV-C treatments (P<0.05).

three pairs of legs, the strongly affected level was found at 8 min of radiation in forelegs. But in midlegs and hindlegs, exposure time to induce the strongly affected level were found at 16 and 32 min of radiation, respectively. These results indicated that the forelegs were more susceptible to UV-C radiation than midlegs and hindlegs.

The sizes in each segment of *T. castaneum* legs were altered by UV-C radiation. The adult had stout legs that extended laterally, and each segment is shorter but broader. The femur of the resulting adults was reduced in size, especially in the strongly affected level, while the tibia, tarsus and claws were completely deleted. These results suggest that the legs of resulting adults are significantly undersized due to the shortening of mainly the tibia and tarsus. Taken together, UV-C radiation during the pupal stage dramatically interfered with leg development.

UV-C radiation has a clear deformity effect on the leg parts of *T. castaneum*. Our previous studies have reported that UV-C radiation causes severe abnormalities on the elytra and hindwings and specific malformations in the antennae and antennal sensilla of *T. castaneum*. The antennae were greatly reduced in size overall. The trichod sensilla was shorter and broader, while the basiconic sensilla increased in width (Tungjitwitayakul *et al*. 2019, 2020). Our findings are similar to those of the study in the lesser mealworm: UV radiation of *Alphitobius diaperinus* caused higher numbers of adult deformities at long exposure periods (Furaki *et al*. 2005). The impact of UV-C radiation was equivalent to gamma irradiation as reported in alfalfa weevils, *H. postica*. When pupae of alfalfa weevils irradiated with gamma-ray, emerged adults had abnormal antennae, legs, and elytra (Burges & Bennett 1971, 1972). Haiba (1998) noticed that gamma irradiation had caused highly significant and incomplete claw development in the midleg of males' potato tuber moth, *Phthorimaea operculella* (Zeller, 1873). The inner side of the gamma-irradiated male claw of *Ceratitis capitata* was broken and showed a saw-like structure from their inner side as compared to control (El-Akhdar *et al*. 2005). The effect of gamma irradiation reflects a clear morphological change in claws, spurs, and scales in *S. littoralis* (El-Degwi & Garbaty 2015). From the above information, UV-C and gamma irradiation also induced undesirable effect on most of the insect legs, which might lead to that inability to grip on rough surfaces according to claw deformities, hold female during mating due to spurs abnormalities, and legs became weaker and lost their protection due to the fallen scales (El-Degwi & Gabarty 2015). Accordingly, irradiated red flour beetle with deformed legs, especially at the claws, might have similar issues leading to low potential of feeding, walking, and reproduction. However, the effects of UV-C on the reproduction of *T. castaneum* need to be

studied in the future.

Differences in the lengths and widths of trichoid sensilla were recorded in the resulting adult of UV-C treated *T. castaneum* pupae. In the moderately affected level, UV-C rays increased the length and width of the trichoid sensilla on the femur but decreased the length and width of the trichoid sensilla on the tibia. Disorganization of trichoid and chaetica sensillae is clearly observed in the pretarsus segment of the legs in *S. littoralis* after gamma radiation (El-Degwi & Garbaty 2015, Hazaa *et al*. 2006). Since the trichoid sensilla have an olfactory function (Hallberg *et al*. 2003), then the change in sizes of trichoid sensilla is expected alter the ability in the odor reception and mechanoreceptors and lead to the disruption of feeding preferences, survival, and environment adaptation in *T. castaneum*.

UV-C radiation not only exerts its effects on the morphology of appendages, including antennae, wings, and legs, but it altered the expression level of heat shock protein genes in *Sitophilus zeamais* (Tungjitwitayakul *et al*. 2016). Taken together, it is possible that UV-C radiation disrupts the appendage patterning genes in *T. castaneum*, such as *Sp8*, *Distall-less* (*Dll*), *nubbin* (*nub*), and *Wnt-1* signaling genes, as reported in several studies (Beermann *et al*. 2004, Suzuki *et al*. 2009, Shah *et al*. 2011, Turchyn *et al*. 2011). Therefore, the effect of UV-C on the expression level of genes involved in appendage patterning will be examined in future work.

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References

Baden HP, Kollias N, Anderson RR, Hopkins T, Raftery L. 1996. *Drosophila melanogaster* larvae detect low doses of UVC radiation as manifested by a writhing response. *Archives of Insect Biochemistry and Physiology*, 32: 187–196.

Beermann A, Aranda M, Schroder R. 2004. The Sp8 zinc-finger transcription factor is involved in allometric growth of the limbs in the beetle *Tribolium castaneum*. *Development*, 131: 733–742.

Burgess EE, Bennet SE. 1971. Mortality and abnormalities caused by gamma irradiation of alfalfa weevil larvae. *Annals of the Entomological Society of America*, 64: 88–90.

Burgess EE, Bennet SE. 1972. Mortality and abnormalities caused by gamma irradiation of alfalfa weevil pupae. *Annals of the Entomological Society of America*, 65: 1331–1333.

De Boer G. 2006. The role of the antennae and maxillary palps in mediating food preferences by larvae of the tobacco hornworm *Manduca sexta*. *Entomologia Experimentalis et Applicata*, 119: 29–38.

Debry CD, Steullet P. 2001. Why do animals have many receptors? The role of multiple chemosensors in animal perception. *Biological Bulletin*, 200: 211–215.

El-Akhdar EAH, Ahmed ZA, Haiba IM. 2005. Effect of gamma irradiation on the fine morphology of legs in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) *Arab Journal of Nuclear Sciences and Applications*, 37(2): 301.

El-Degwi MS, Gabarty A. 2015. Morphological changes induced by thermal treatment and gamma irradiation on the males' hind legs of *Spodoptera littoralis* (Noctuidae; Lepidoptera). *Journal of Radiation Research and Applied Sciences*, 8: 508–515.

Espo E, Eyidozehi K, Ravan S. 2015. Influence of gamma and ultraviolet irradiation on pest control. *The Museums and Art Galleries of the Northern Territory Research Report*, 3(2): 319–326.

Furaki SI, Das DR, Khatun M. 2005. Effects of UV-radiation on the larvae of the lesser mealworm, *Alphitobius diaperinus* (Panzer)

(Coleoptera: Tenebrionidae) and their progeny. *Journal of Biological Sciences*, 5: 444–448.

Haiba IM. 1998. Morphological effect of gamma radiation on the legs of potato tuber moth, *Phthorimae operculellaea* (Zeller) (Lepidoptera: Gelechiidae). *Arab Journal of Nuclear Sciences and Applications*, 31(2): 301–316.

Hallberg E, Hansson BS, Löfstedt C. 2003. Sensilla and proprioceptors. In: Kristensen N. (Ed.). *Lepidoptera*, *moths and butterflies*, *Handbook of zoology*, IV, Vol. 2, de Gruyter, Berlin, pp. 267–288.

Hazaa MAM, El-Shall SSA, Alm El-Din MMS. 2006. Scanning electron microscope studies of an olfactory sensillum on the leg of gamma irradiated *Spodeptera littoralis* adults. *Isotope and Radiation Research*, 38(1): 165–82.

Li Z, Zhang Y, Wang Q, Khashaveh A, Gu S, Liu S, Zhang Y. 2020. Identification of leg chemosensory genes and sensilla in the *Apolygus lucorum*. *Frontiers in Physiology*, 11: 1–15.
DOI: 10.3389/fphys.2020.00276.

Lu HH, Zhou JC, Yan D, Zhao SM, Xiong SB. 2011. Effects of microwave radiation and conductive heating on *Tribolium castaneum* microstructure. *Micron*, 42: 36–41.

Seada MA, Hamza AM. 2018. Differential morphology of the sensory sensilla of antennae, palpi, foretarsi and ovipositor of adult *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Annals of Agricultural Sciences*, 63(1): 1–8.

Shah MV, Namigai EKO, Suzuki Y. 2011. The role of canonical Wnt signaling in leg regeneration and metamorphosis in the red flour beetle *Tribolium castaneum*. *Mechanisms of development*, 128: 342–358.

Skiri HT, Stranden M, Sandoz JC, Menzel R, Mustaparta H. 2005. Associative learning of plant odorants activating the same or different receptor neurones in the moth *Heliothis virescens*. *Journal of Experimental Biology*, 208: 787–796.

Smith FW, Anlelini DR, Jockusch EL. 2014. A functional genetic analysis in flour beetles (Tenebionidae) reveals an antennal identity specification mechanism active during metamorphosis in Holometabola. *Mechanisms of Development*, 132: 13–27.

Suzuki Y, Squires DC, Riddiford LM. 2009. Larval leg integrity is maintained by Distal-less and is required for proper timing of metamorphosis in the flour beetle, *Tribolium castaneum*. *Developmental Biology*, 326: 60–67.

Tungjitwitayakul J, Tatun N, Vajarasathira B, Sakurai S. 2016. Effects of ultraviolet-C and microwave irradiation on the expression of heat shock protein genes in the maize weevil (Coleoptera: Curculionidae). *European Journal of Entomology*, 113: 135–142. DOI: 10.14411/eje.2016.017

Tungjitwitayakul J, Yasanga T, Tatun N. 2019. UV-C radiation during the pupal stage affects morphological changes of wings in *Tribolium castaneum* (Col; Tenebrionidae). *International Journal of Radiation Biology*, 95(9): 1309-1318.
DOI:10.1080/09553002.2019.1625492.

Tungjitwitayakul J, Yasanga T, Tatun N. 2020. Impact of UV-C radiation on morphology of the antenna and antennal sensilla in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Radiation Research and Applied Sciences*, 13(1): 648-656.
DOI: 10.1080/16878507.2020.1812797.

Turchyn N, Chesebro J, Hrycaj S, Couso JP, PopadiĆ A. 2011. Evolution of *nubbin* function in hemimetabolous and holometabolous insect appendages. *Developmental Biology*, 357: 84–95.

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