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Research article

Differences in Climbing Ability of *Cimex lectularius* and *Cimex hemipterus* (Hemiptera: Cimicidae)

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Abstract

The climbing abilities of two bed bug species, *Cimex lectularius* L. and *Cimex hemipterus* (F.), were determined by evaluating their escape rates from smooth surface pitfall traps using four commercial bed bug monitors (Verifi Bed Bug Detector, ClimbUp Insect Interceptor, BlackOut Bed Bug Detector, and SenSci Volcano Bed Bug Detector). All detectors were used in the absence of lures or attractants. Unlike *C. lectularius*, adult *C. hemipterus* were able to escape from all traps. On the other hand, no or a low number nymphs of both species escaped, depending on the evaluated traps. Examination of the vertical friction force of adults of both species revealed a higher vertical friction force in *C. hemipterus* than in *C. lectularius*. Scanning electron microscope micrograph observation on the tibial pad of adult bed bugs of *C. hemipterus* showed the presence of a greater number of tenent hairs on the tibial pad than on that of adult *C. lectularius*. No tibial pad was found on the fourth and fifth instars of both species. Near the base of the hollow tenent hairs is a glandular epithelium that is better developed in adult *C. hemipterus* than in adult *C. lectularius*. This study highlights significant morphological differences between *C. lectularius* and *C. hemipterus*, which may have implications in the monitoring and management of bed bug infestations.

Key words: bed bug, tenent hair, vertical friction force, climbing, pitfall trap

The current global resurgence of bed bugs involves two species, namely the common bed bug, *Cimex lectularius* L. and the tropical bed bug, *Cimex hemipterus* (F.). *Cimex lectularius* is mainly found in the temperate and subtropical regions, while the latter species is distributed in the subtropical and tropical regions (Omori 1939, 1941, Usinger 1966, Doggett et al. 2003, How and Lee 2010, Zulaikha et al. 2016). In some regions such as in Africa (Newberry 1988, 1989), Australia (Doggett et al. 2003), Florida (Hixson 1943, Campbell et al. 2016), and Taiwan (Lee 2013), both species can coexist sympatrically.

The detection and monitoring of bed bugs is one of the most important aspects of integrated pest management (IPM) against this insect (Wang and Cooper 2011). Detection and monitoring of bed bugs can be undertaken via visual inspections, through various traps and monitoring devices, via canine detection (Pinto et al. 2007, Wang and Cooper 2011, Boase and Naylor 2014), or any combination of these. Amongst the monitors available in the market are pitfall and sticky traps, some with the addition of lures such as heat, carbon dioxide, and insect or host odors (active monitors), while others contain no lure (passive monitors) and provide a harborage for bed bugs to enter. Although many such products exist in the market, few have been tested for efficacy via independent scientific tests.

Pitfall style traps of different designs have been found effective in detecting and monitoring *C. lectularius* (Singh et al. 2013). These traps rely on a smooth inner wall surface to prevent trapped bed bugs from escaping. Such traps with smooth surfaces can also be used as a barrier to prevent bed bugs from reaching the sleeping hosts (Doggett 2013). However, if the trapped bugs were able to climb up the smooth surfaces and escape, this would seriously compromise monitoring efforts or their use as barriers. It is known that a buildup of dust and debris can compromise the efficacy of pitfall traps against *C. lectularius*, and hence, maintenance recommendations are usually included, such as regular cleaning or the addition of talc (Doggett 2013).

Most of the trap products were originally developed and tested in the United States, where *C. lectularius* is the major bed bug species. Many of these products have subsequently been marketed in other parts of the world on the assumption that they also would work against the species that were found there, such as

C. hemipterus. In a preliminary laboratory investigation though, it was observed that adult *C. hemipterus* could escape from a new pitfall trap, which otherwise effectively contained *C. lectularius*. This raised an important question: are all pitfall traps that are effective in containing *C. lectularius* also effective against *C. hemipterus*? If differences are found, this would have profound implications for the monitoring of the latter species and for the potential use of barriers against *C. hemipterus*. Furthermore, it would raise the question why such differences exist. The results of the initial investigation prompted the following study. The escape rate was compared between *C. lectularius* and *C. hemipterus* in four commercially available monitors that were previously or presently marketed for bed bug detection and monitoring in the United States. Following this, the vertical friction force was determined for both species on two types of surfaces (smooth and rough). Lastly, the leg morphology was investigated to determine the possible reasons behind the differences in climbing ability of both species.

Materials and Methods

Insects

Two to three strains for each species were used; *C. lectularius* (Monheim [MH] and Sydney [SYD] strains), and *C. hemipterus* (Kuala Lumpur [KL], Queensland [QSL], and Greenlane [GL] strains; Table 1). All strains were reared in the laboratory in glass jars (7 cm in diameter × 9 cm in height) and provided with folded brown paper as harborage under environmental conditions of 26 ± 2°C, 70 ± 5% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. All the insects used in this study were blood-fed on a human volunteer once a week. An alcohol-preserved sample of adult *C. hemipterus* collected from Papua New Guinea in 1932 [PNG strain] was used in part of the scanning electron microscope (SEM) observation to examine if any morphological change could have been a recent evolutionary adaptation owing to human management interventions, such as the use of residual organic insecticides.

Trap Escape Test

The insects were evaluated in their ability to escape from four bed bug pitfall traps namely, Verifi bed bug detector (FMC Professional Solutions, Philadelphia, PA), ClimbUp insect interceptor (Susan McKnight Inc, Memphis, TN; without the talc), BlackOut bed bug detector (Protect-A-Bed, Wheeling, IL), and SenSci Volcano bed bug detector (Bedbug Central, Lawrenceville, NJ). All traps were cleaned with 70% ethanol and dried in a 60°C incubator overnight before the test. Although ClimbUp traps are normally used with the talc (as per the manufacturer's recommendations), they were evaluated in the absence of talc in this study. Lures are available for use in conjunction with the Verifi and Volcano traps to enhance their collection efficacy but they were not used in this study. One strain of each species was tested: *C. lectularius* (SYD strain) and *C. hemipterus* (KL strain). The evaluation was carried out by introducing 5 adult males, 5 adult females and 5 fourth to fifth instars into the trap. The tested insects were placed in the pitfall for both Verifi and Volcano traps, and in the outer well for the ClimbUp and Blackout traps. The traps were placed inside test arenas (50 cm in length × 30 cm in width × 10 cm in height) and kept in an environmental chamber (Binder Model KBF 240, Binder GmbH, Tuttlingen, Germany) under conditions of 26 ± 1°C, 70 ± 1% RH, and a 24-h scotophase. All these insects were blood-fed 5 d prior to the test. The experiments began at 2000 hours, and at 4-, 24-, 48- and 72-h post treatment, the number of insects that remained in the trap, and the number that escaped were counted. Observations were made up to 72 h for all traps except for the Volcano trap which was observed daily for up to 10-d post treatment. All experiments were replicated three times.

Vertical Friction Force Measurement

To confirm that the differences in climbing ability of the insects within the pitfall traps was owing to better holding ability on the smooth surface of one species over the other, the vertical friction forces of both *C. lectularius* and *C. hemipterus* were measured with a Sartorius ED224S (Sartorius, Göttingen, Germany) electronic balance using modified methods described by Betz (2002) and Hottel et al. (2015). A data recording program, Sartorius Weight Anchor Software (Sartorius, Göttingen, Germany) installed on a computer, was connected to the electronic balance using a Sartorius RS232 cable (Sartorius, Göttingen, Germany). A bed bug was tethered to a polyethylene bristle by gluing (UHU Power Glue, UHU GmbH & Co. KG, Bühl, Germany) its dorsal side to one tip of the bristle. The other tip of the bristle was inserted into a 30-g ball of modelling clay (Tack-it, A.W. Faber-Castell (India) Ltd, Mumbai, India). The clay ball, which was attached to the bristle, was placed on the balance with the tethered bed bug over the other end, and tared to zero.

Two surfaces were tested, namely a smooth surface (glass microscope cover slide), and a rough surface (filter paper). The test surface was moved to the tethered bed bug to permit the insect's legs to come into contact. Once the insect had contacted and gripped onto the test surface, the software started recording the changes in the mass of the clay ball for 5 min. The data was then converted from mass (g) to force (μN) using the formula, $F = ma$, where $m =$ mass (g) and $a =$ acceleration $= -9.81$ m^2/s. The mean vertical friction force (MVFF) was calculated using the top five maximum readings. One strain of each species was tested: *C. lectularius* (SYD strain) and *C. hemipterus* (KL strain). Five adult males and five adult females were used for each species. All these insects were blood-fed 5 d prior to the test. The weight of the insects (g) was individually measured using the balance, and converted to μN using the formula as stated above. The MVFF: insect weight ratio was calculated and used to compare between both species, instead of using MVFF to equalize the effect of weight differences between the species.

Table 1. The bed bug strains used in this study

Species	Strain	Year established	Remarks
C. hemipterus	Greenlane (GL)	2015	Field collected
	Kuala Lumpur (KL)	2005	Lab colony
	Queensland (QSL)	~2004	Lab colony
	Papua New Guinea (PNG)	1932	Curated specimens in 70% ethanol
C. lectularius	Monheim (MH)	~1970	Lab colony
	Sydney (SYD)	2004	Lab colony

Scanning Electron Microscope Observation of the Tibial Pad (Fossula Spongiosa)

Five adult males and five adult females of the two species (*C. lectularius* [MH and SYD strains], and *C. hemipterus* [KL, GL, and PNG strains]) were used for SEM observation of the tibial pad. Two each of fourth instars and fifth instars of *C. lectularius* (MH strain) were examined for the presence of the tibial pad. Bed bugs were preserved in 70% ethanol, dehydrated, and cleaned using acetone and a Sonicator SC-52H (Sonicor Instrument Corp, New York, NY). The fore-, mid-, and hind-legs of the specimens were excised and mounted on an aluminum stub. The specimens were coated with gold and observed under a scanning electron microscope (SEM) at 15 kV (Leo Supra 50VP field emission SEM, Carl Zeiss SMT, Oberkochen, Germany). The number of tenent hairs (setae) on the tibial pad of each leg was counted from the SEM images taken from different angles.

Histological Examination of the Tibial Pad in *C. lectularius* and *C. hemipterus*

The tibiae with attached tarsomeres of the six legs of three male and three female adults of both *C. lectularius* (MH strain) and *C. hemipterus* (QSL strain) were cut off with microsurgery scissors, and fixed in cold 2% glutaraldehyde, buffered with 50 mM Na-cacodylate and 150 mM saccharose. Tissues were postfixed in 2% osmium tetroxide in the same buffer, dehydrated through a graded acetone series, and embedded in Araldite resin. Serial semi-thin sections with a thickness of 1 µm were made with a Leica EM UC6 ultramicrotome (Leica Biosystems, Nussloch, Germany), stained with methylene blue and thionin, and examined with an Olympus BX-51 light microscope (Olympus Corporation, Tokyo, Japan).

Statistical Analysis

Data in percentage of escaped bed bugs from the traps were converted to arc-sine values before the analyses. A *t*-Test was used to compare between the trap escape rates of *C. lectularius* and *C. hemipterus*. Data on vertical friction force and the number of tenent hairs for adult males and females of both species were subjected to one-way analysis of variance (ANOVA), and means were separated using Tukey's HSD at 95% confidence level. All analyses were performed using the statistical program SPSS version 20 (IBM Corp., Armonk, NY).

Results

Trap Escape Test

Results showed that all stages tested of *C. hemipterus* were able to escape from each of the traps, with mean combined escape rates ranging from 24.4 ± 4.4% to 75.6 ± 4.4% (Fig. 1) per trap, at 72-h post treatment. In contrast, most traps were effective in containing *C. lectularius* with only 0–2.2% escape rates, except for the ClimbUp insect interceptor which registered a 26.7 ± 10.2% mean escape rate. The escape rates from all traps were significantly different ($P < 0.05$) between *C. lectularius* and *C. hemipterus*. For the Volcano trap, an observation of up to 10 d revealed an escape rate of 55.0 ± 27.5% for *C. hemipterus*, while no *C. lectularius* escaped during the corresponding period.

When the monitors were tested against adults of *C. hemipterus*, the mean cumulative percentage of escaped insects in Verifi and ClimbUp traps were significantly higher ($P < 0.05$) than the BlackOut and the Volcano traps at 4-h post treatment (Fig. 2). Regarding *C. lectularius*, significantly ($P < 0.05$) more adults escaped from the ClimbUp trap than from the other three evaluated traps. No nymphs of either species were able to escape from the Blackout and Volcano traps (Fig. 2). For *C. lectularius*, no nymphs escaped from Verifi trap, but 13.3 ± 6.7% nymphs escaped from ClimbUp trap at 72-h post-treatment. When tested against adult *C. lectularius*, 3.3 ± 3.3% and 33.3 ± 12.0% adults escaped from Verifi and ClimbUp traps, respectively. Despite some nymph escapees, there were no significant differences ($P > 0.05$) in the mean cumulative percentage of escaped nymphal bed bugs of all four evaluated traps. Both Blackout and Volcano traps were effective in containing the *C. lectularius* adults, with no escapees throughout the 72-h evaluation period (as well as at 10 d post treatment for Volcano trap).

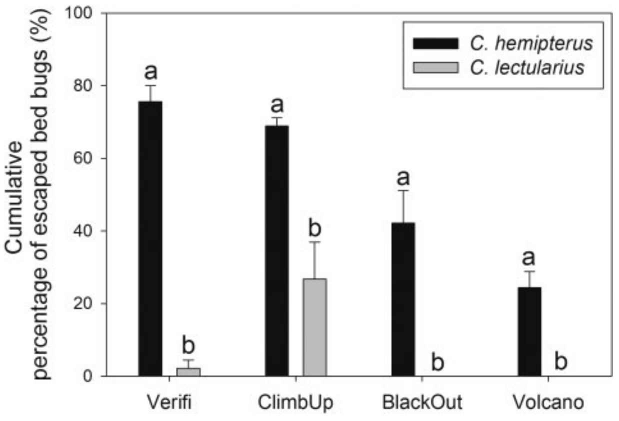

Fig. 1. Cumulative percentage of combined escaped adults and nymphs of bed bugs (Mean ± SEM) from the four traps after 72-h post treatment. Letters above each bar are assigned for comparison between the same trap and different letters denote significant difference ($P < 0.05$, *t*-test).

Nymphs of *C. hemipterus* managed to escape from Verifi and ClimbUp traps at the rates of 26.7 ± 13.3% and 20.0 ± 11.5%, respectively, at 72 h. There were no nymph escapees for the Blackout and Volcano traps. Verifi was the least efficient trap against the adults of *C. hemipterus*, with 100% escapees within 4 h after the bed bugs were introduced into the trap. ClimbUp trap also showed a high escape rate (>90%) for *C. hemipterus* adults at 72-h post treatment. In contrast, 60.0 ± 15.3% and 36.7 ± 6.7% of adult *C. hemipterus* escaped from Blackout and Volcano traps, respectively, during the same 72-h evaluation period.

Vertical Friction Force

Visual observation revealed that when bed bugs were climbing on the rough surface, the tarsal claws were used, whereas tibial pads only were used while climbing on smooth surfaces (Fig. 3). In this study, *C. hemipterus* demonstrated a significantly higher vertical friction force ($P < 0.05$) of ~3–4× that of *C. lectularius* when tested on smooth glass microscope cover slides (Table 2). However, there appeared to be no differences in vertical friction force in both species when the insects were tested on filter paper as a rough substrate. No differences in vertical friction force were found between adult males and females of each species.

Scanning Electron Microscope Observation on Tibial Pad

Tibial pads were present in the adults of both species, but they were not present in the fourth instar (Fig. 4) and fifth instar. The pads appear as an oval disk of ~120 by 40 µm at the ventrodistal

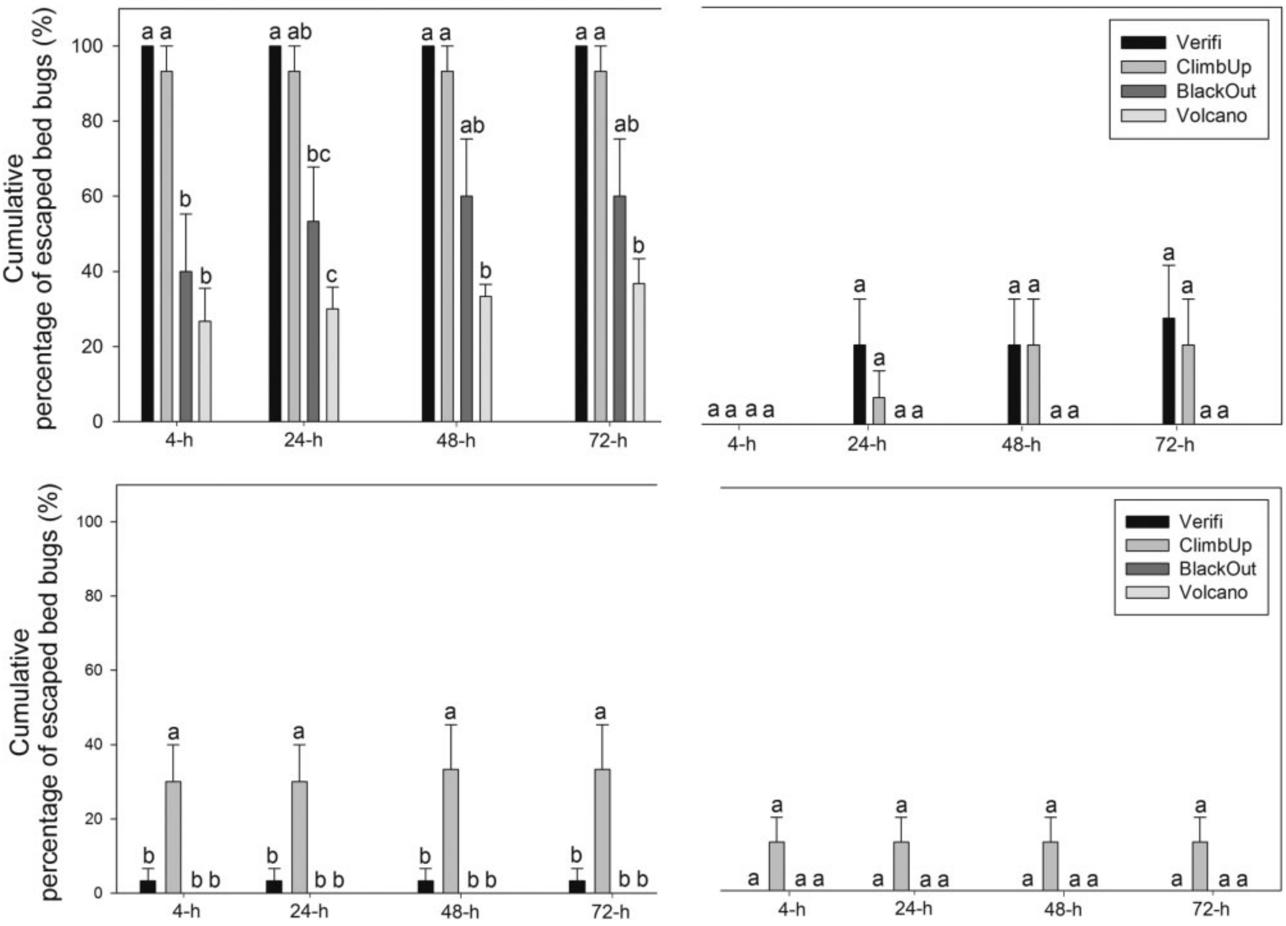

Fig. 2. Mean cumulative percentage of escaped bed bugs ± SE at 4-, 24-, 48-, and 72-h post treatment (Top left: *C. hemipterus* adults; Top right, *C. hemipterus* nymphs; Bottom left, *C. lectularius* adults; Bottom right, *C. lectularius* nymphs). Letters above each bar are assigned for comparison between the traps and different letters denote significant difference ($P = 0.05$, Tukey's HSD).

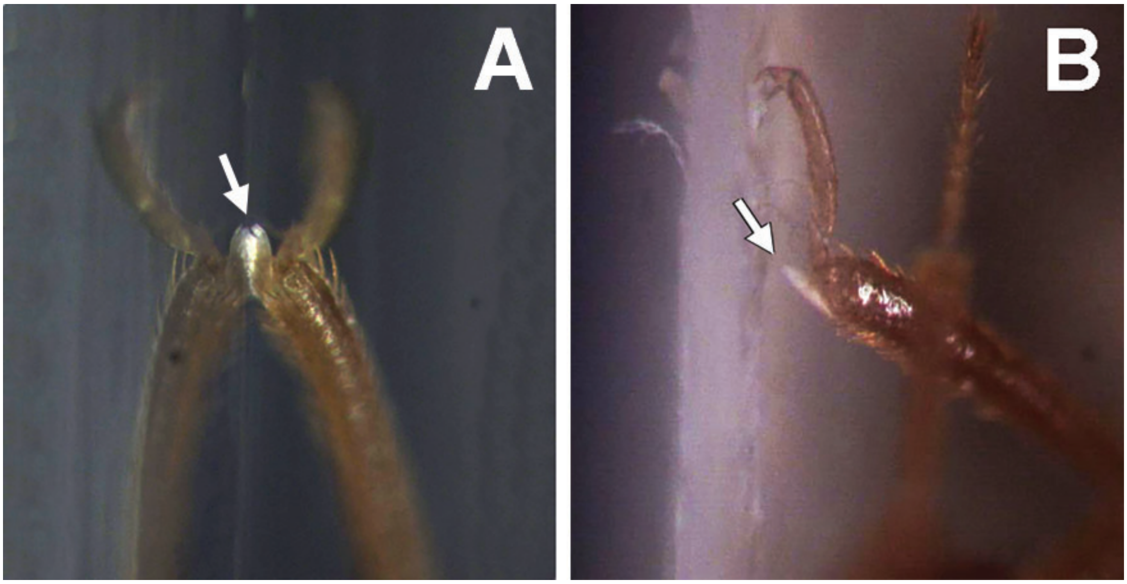

Fig. 3. Detail of fore-leg of *C. hemipterus* male climbing on plastic petri dish (smooth surface) (**A**) and filter paper (rough surface) (**B**). Note that whitish tibial pad (arrow) is in contact with substrate only on smooth surface. Observation was made using live bed bugs under an SZ61 stereomicroscope (Olympus, Tokyo, Japan) connected to a CCD camera (color online, black and white in print).

Table 2. Mean vertical friction forces (MVFF) on smooth and rough surfaces, weights, and MVFF: weight ratios of the adult male and female of *C. lectularius* and *C. hemipterus*

Species	Sex	MVFF ± SE (μN)[a]		weight (μN)	MVFF: weight ratio	
		Cover glass	Filter paper		Cover glass	Filter paper
C. hemipterus	M	156.8 ± 9.2a	2476 ± 140.5a	25.5 ± 2.5c	6.3 ± 0.6a	102 ± 13.4a
	F	184.8 ± 37.8a	3203 ± 579.5a	30.6 ± 2.0bc	6.5 ± 1.7a	110 ± 25.0a
C. lectularius	M	48.7 ± 10.2b	2338 ± 307.3a	37.9 ± 2.4ab	1.3 ± 0.3b	62 ± 6.7a
	F	42.2 ± 11.8b	3181 ± 516.0a	43.6 ± 4.1a	1.0 ± 0.2b	77 ± 17.9a

[a] Means followed by different letters within the same column are significantly different ($P < 0.05$; Tukey's HSD).

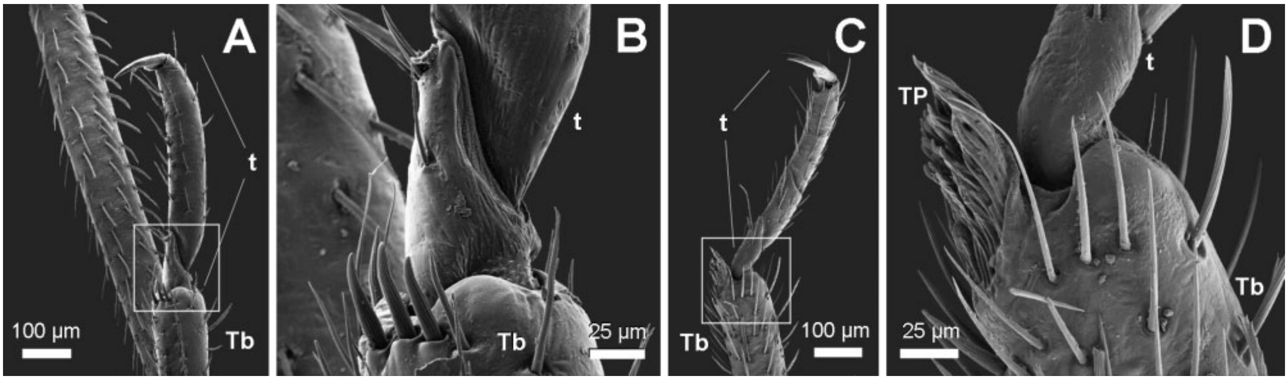

Fig. 4. Scanning electron microscope micrographs showing the absence of tibial pad in fourth-instar *C. lectularius* (**A**, **B**), and presence of tibial pad in adult female *C. lectularius* (**C**, **D**). The framed area in A and C indicate the parts that are enlarged in B and D, respectively. t: tarsomeres; Tb: tibia; TP: tibial pad.

portion of the tibia of the six legs (Fig. 5). The ventral side of the pad is covered with parallel tenent hairs that have a golf club-like appearance. The hairs have a length between 30 and 40 μm and a diameter around 2 μm, the club-like distal portion having a length of ∼10 μm (Fig. 5). The number of tenent hairs on the tibial pad of *C. hemipterus* was significantly higher ($P < 0.05$) than in *C. lectularius* (Table 3, Fig. 5). The number of tenent hairs appeared to be similar among the different strains of each species (Table 3); however, the fore- and mid-legs showed more tenent hairs than the hind-legs. The three strains of *C. hemipterus* have almost twice the number of tenent hairs ($P < 0.05$) on the hind-legs, compared with *C. lectularius*. The adult male of *C. lectularius* had significantly more ($P < 0.05$) tenent hairs than the adult female of the same species on their fore- and mid-legs, but this observation was not recorded for *C. hemipterus*. The rank in total tenent hairs on the fore-, mid- and hind-legs for one side of the body is as follows: *C. hemipterus* males = *C. hemipterus* females > *C. lectularius* males > *C. lectularius* females.

Histological Examination of Tibial Pad in *C. lectularius* and *C. hemipterus*

Serial longitudinal sections through the distal tibial part revealed details of the internal anatomy of the tibial pad region (Fig. 6A–D). The tenent hairs are hollow with an internal diameter around 1 μm, and penetrate the cuticular plate that forms the base of the tibial pad. In all legs of both males and females of *C. lectularius* and *C. hemipterus*, the tegumental epithelium of the tibia is differentiated into a glandular epithelium. This epithelium in all legs is more pronounced in *C. hemipterus*, where it reaches a thickness of 30 μm, while in *C. lectularius*, it is approximately half as thick (Fig. 6A–D). At its apical side, the epithelium shows a subcuticular space in which its secretory products can be stored, and which is continuous with the central space of the hollow tenent hairs. The epithelium is not associated with any muscular tissue. The tibia contains two tendons, however, of which the ventral one passes in the near vicinity of the glandular epithelium. Both tendons are connected with muscles in the proximal part of the tibia and distally to the basitarsus, of which they direct the movements.

Discussion

Unlike *C. lectularius*, adult *C. hemipterus* were found to escape from all pitfall traps evaluated. Examination of the vertical friction force of both species revealed higher vertical friction forces in *C. hemipterus*, compared with *C. lectularius*. As bed bugs climb on rough surface using their tarsal claws, and on smooth surfaces using the tibial pads, it was suspected that the differences in climbing ability between the two species must be related to variations in the tibial pad. Although Wigglesworth (1938) previously reported that the tibial pad did not assist in the climbing of smooth surfaces by the bed bug, he was experimenting using only *C. lectularius*. Should he have worked on *C. hemipterus*, his conclusion would have been very different. In *Triatoma* spp., it has been proposed that the tibial pads enable the adult bugs to climb smooth surfaces (Gillett and Wigglesworth 1932, Weirauch 2007). Usinger (1966) described the presence of the tibial pad in *C. lectularius*, but did not indicate its presence in *C. hemipterus*.

To date, the tibial pad had been reported as "fossula spongiosa" by Haridass and Ananthakrishnan (1980), "apex of tibia" by Walpole (1987), and "tibial brush" by several authors (Ferris and Usinger 1957, Baker et al. 2016). The tenent hairs (Haridass and

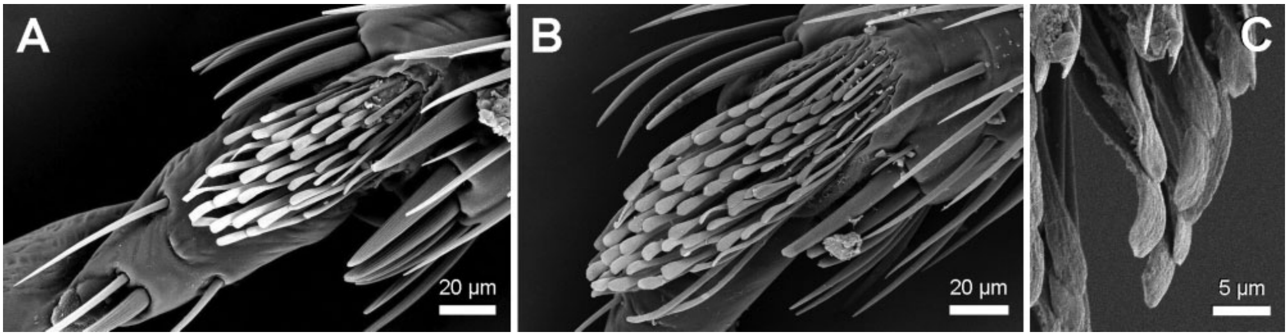

Fig. 5. Scanning electron micrographs showing the tibial pad of the hind-legs of an adult female in *C. lectularius* (**A**) and *C. hemipterus* (**B**). **C.** Detail of tenent hairs of male *C. hemipterus* mid-leg.

Table 3. Mean number of tenent hairs on tibial pad of the fore-, mid-, and hind-legs of adult *C. hemipterus* and *C. lectularius*

Species	Sex	Strains	Fore-leg[a]	Mid-leg[a]	Hind-leg[a]	Total[a]
C. hemipterus	M	KL	117.9 ± 5.7a	107.1 ± 6.0a	86.2 ± 2.5a	335.7 ± 13.6a
		GL	101.8 ± 2.6ab	97.6 ± 3.4a	78.8 ± 2.5a	292.2 ± 9.2ab
		PNG	113.1 ± 2.9a	106.4 ± 2.7a	79.3 ± 2.4a	318.2 ± 8.1a
	F	KL	100.0 ± 8.1ab	112.7 ± 3.1a	88.1 ± 1.9a	309.7 ± 11.8a
		GL	105.7 ± 4.3a	110.3 ± 3.4a	87.7 ± 2.9a	328.6 ± 9.9a
		PNG	115.9 ± 3.6a	115.7 ± 4.2a	89.8 ± 1.7a	340.6 ± 10.0a
C. lectularius	M	MH	119.8 ± 7.3a	99.3 ± 5.4a	41.8 ± 2.2b	247.6 ± 10.4bc
		SYD	114.2 ± 5.3a	102.7 ± 3.9a	49.2 ± 2.0b	239.4 ± 12.2c
	F	MH	66.8 ± 0.7c	71.6 ± 1.4b	43.2 ± 2.4b	176.8 ± 4.0d
		SYD	79.6 ± 1.7bc	78.0 ± 3.9b	47.8 ± 1.9b	200.4 ± 7.0cd

[a] Means followed by different letters within the same column are significantly different ($P < 0.05$, Tukey's HSD).

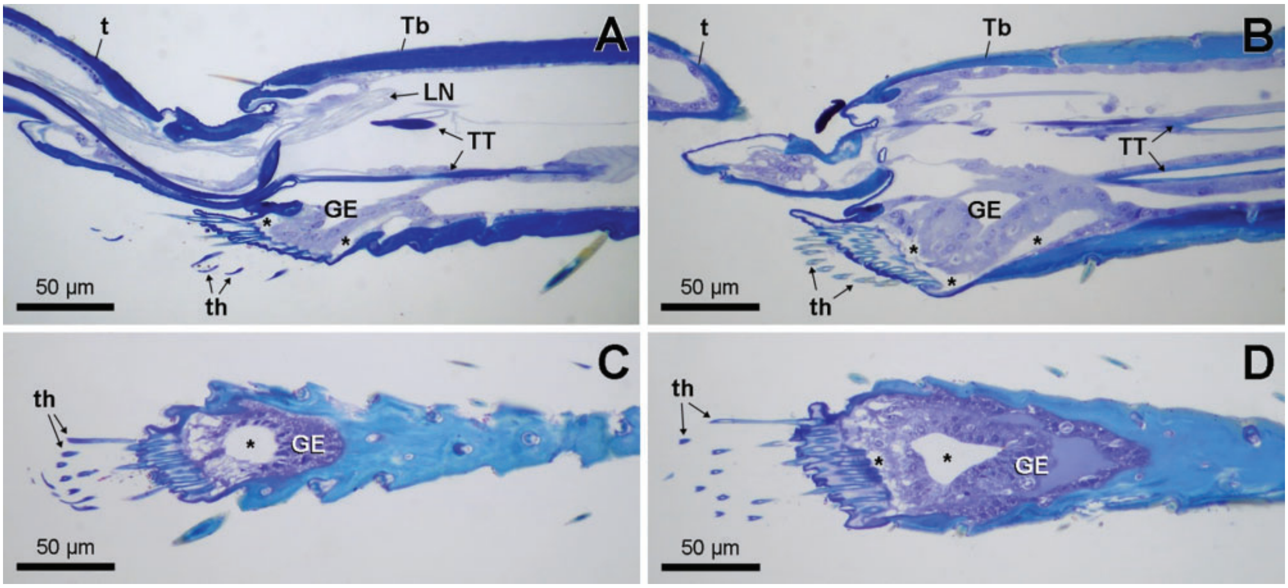

Fig. 6. Longitudinal sections through the tibial pad of *C. lectularius* (**A**, male mid leg) and *C. hemipterus* (**B**, male fore-leg), showing the glandular epithelium (GE), the subcuticular space (asterisks), and the hollow tenent hairs (th). Note the tibia contains two tibial tendons (TT), of which the ventral is close to the glandular epithelium. The sectioning plane in **C** (*C. lectularius* female hind-leg) and **D** (*C. hemipterus* male mid-leg) is perpendicular to that in A and B, and is more or less parallel to the ventral surface of the tibia. LN, leg nerve; t, tarsomeres; Tb, tibia. The histological organization in all legs is similar, the photographs shown in this figure were selected as they were the best available images **(color online, black & white in print)**.

Ananthakrishnan 1980) were described as a “turf of hair” (Usinger 1966), “tibial brush setae” (Baker et al. 2016), or “setae of tibial apex” (Walpole 1987).

Scanning electron microscope observation on the tibial pad revealed a greater number of tenent hairs in *C. hemipterus* than in *C. lectularius*. By incapacitating the function of tenent hairs on the tibial pad using glue, it was found that this affected the mating process of *C. hemipterus*, which led to a reduction in reproductive success (D.-Y.K. & C.-Y.L, unpublished data). It is speculated that the tibial pad may play an important role in the reproduction of the bed bug, beyond that of providing grip in the climbing of smooth surfaces. This argument is further supported by the absence of tibial pads in the nymphal stages, which explains their poor climbing ability as documented in this study. Using SEM, Walpole (1987) too reported that the tibial pad could only be found in the adults of *C. lectularius* and *C. hemipterus*, but failed to determine its function. In other insect orders such as Blattodea (Clemente and Federle 2008), Diptera (Bauchhenss 1979, Gorb 1998, Gorb and Beutel 2001), Hymenoptera (Dirks and Federle 2011), Coleoptera (Ishii 1987, Geiselhardt et al. 2011, Hosoda and Gorb 2011), as well as other families in the order Hemiptera (Gillett and Wigglesworth 1932, Edwards and Tarkanian 1970, Haridass and Ananthakrishnan 1980), the use of the tenent hairs for surface adhesion has been documented. Baker et al. (2016) described the tenent hairs on the tibia pad as "tibia brush setae" and also suggested that these setae may be functional during the mating process and for climbing of various surfaces.

Although the precise mechanism of how the tibial pad allows climbing on smooth vertical surfaces remains still unknown, it seems obvious that the hollow tenent hairs play a role in the ability to do so. Their central space is continuous with the subcuticular space of the glandular epithelium that is associated with the tibial pad (Baker et al. 2016), suggesting that a glandular secretion can be released through the hairs. With an internal diameter around 1 μm, mere capillary action may be sufficient for this. In addition to capillarity, the secretion may also be pumped through the hollow hairs, although there is no direct muscular tissue associated with the glandular epithelium. However, there is the ventral tibial tendon, which is situated in proximity to the epithelium, and which may cause pressure onto the epithelium and hence also on the fluid in the subcuticular space. Such pulling action of the tendon may occur during climbing when the tarsomeres need to be moved in order to bring the tibial pad in the right configuration with respect to the substrate. Increased pressure from the tendon onto the glandular epithelium at the same time may then result in fluid being pumped into the tenent hairs. A somewhat comparable situation is known in ants, in which adhesion to smooth surfaces is possible through the arolium that acts as an adhesive pad, which is situated at the tip of the pretarsus of each leg. The arolium is associated with a sac-like epithelial gland that works as a hydraulic system by pumping liquid into the arolium upon contraction of the leg tendon (Federle et al. 2001). In a recent study comparing two ant species with very opposite climbing ability, it was found that both species possessed an arolium gland, although it was only poorly developed in the non-climbing species (Billen et al. 2017). These reports are in line with the observations herein that both *Cimex* species possess a tibial pad, but that the higher number of tenent hairs and the better developed glandular epithelium in *C. hemipterus* allow this species to climb smooth vertical surfaces.

In the experiments described herein, the ClimbUp trap was evaluated without the addition of talc, and *C. hemipterus* was able to easily escape from the monitor. When talc was applied (as per the manufacturer's recommendations), the monitor was able to contain insects of both *C. lectularius* and *C. hemipterus* with minimal escapees (<5%) over a 72-h period (Kim and Lee, unpublished data). Wang and Cooper (2011) reported that regular talc powder reapplications on pitfall-type monitors are required to prevent bed bugs from escaping. Hottel et al. (2015) found that talc powder reduced the vertical pulling force of *C. lectularius* on glass surfaces and the ClimbUp inner wall. In addition, from our experience, the number of trapped escapees could be minimized by placing a small piece of folded corrugated cardboard inside the pitfall trap. However, this would require further efforts, as the harborage would need to be examined during monitoring programs. It is anecdotally known that the performance of pitfall traps declines in use over time owing to the buildup of dust and debris, and can even overcome the addition of talc (Singh et al. 2013, Wang et al. 2013, Cooper et al. 2016). A trial examining variably aged traps could provide insights into the required maintenance intervals to prevent escape of the respective species.

The present study demonstrated the need for the design of a more efficient bed bug trap (and the need to reconsider trap maintenance intervals) that could be used against both *C. lectularius* and *C. hemipterus*. New low-friction surfaces for pitfall traps could also be explored, as long as the cost per unit item for the trap would not financially compromise any monitoring program. It remains unknown at this stage why these sympatric species of bed bugs could have these differences in leg morphology, and the possible biological trade-offs it may have on *C. hemipterus*.

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